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Effects of a hiding opportunity on fURI,
bodyweight and adoption rates in different
housing conditions in Dutch shelter cats



ABSTRACT

The total cat population in the Netherlands consists of circa 2,6 million individuals and annually, approximately 52.000 cats end up at facilities for shelter and reassignments. Failure to cope with these environmental changes can indicate a decline in welfare, leading to immunodeficiency making the cat more vulnerable for upper respiratory infections, reducing body weights and lower adoption chances. Hiding enrichment can play an important role in preventing an impairment in welfare since cats will have an opportunity to remove themselves from stressors. In the current study we aimed to determine an easy measurable and practicable parameter for monitoring stress in shelter cats which can be used by shelter workers and volunteers with the purpose to recognize, reduce and even prevent stress, thereby optimizing health and welfare in shelter cats.

15 newly arrived European short hair cats between 1 and 11 years of age were randomly assigned to two treatment groups: the experimental group ($N = 9$) which had access to a hiding box, and the control group ($N = 7$) which did not have access to a hiding box. Place preference locations were determined for either the first 14 days in individual housing in quarantine area, as for the first 7 days in consecutively social housing units on day 1,2,3,5,7,9,12,14,15,17,19 and 21. Social Adaptation Score (SAS) was introduced in order to relate stress levels to spending time in close proximity to unfamiliar conspecifics in social housing on day 15,17,19 and 21. fURI scoring took place on day 1,2,3,5,7,9,12 and 14 in single housing conditions. Body weights were determined for week 1, 2 and 3 and food- and water intake were registered for days 1 through 14. Number of days between leaving the quarantine area and being adopted by a new owner were registered in order to determine adoption rates.

Most important findings include:

1. Cats without access to a hiding box spent significant more time elsewhere than cats who did have access to a hiding box. More than half of the total observed time was spent in the box. In social housing units, cats spent most of their time outdoors on ground level in peripheral areas followed by a preference for being in an enrichment item with hiding purpose. Central areas were least favorite.
2. Significant differences in bodyweight were observed between day 1 and day 7, and between day 1 and day 21. 13 out of 16 cats lost weight during the first 14 days in shelter and 5 out of 8 cats continued losing weight after leaving the quarantine area. Although, there were no indications for a significant difference between the treatment groups regarding to bodyweight losses, mean overall bodyweight loss in the control group (-4,7%) was clearly more pronounced as in the experimental group (-2,9%).
3. 100% of included cats had or developed signs of fURI during the first 14 days in the shelter. And higher fURI scores were significantly and positively correlated with older cats.
4. A SAS of 1 was noted for 2 out of 5 cats.
5. No significant differences were detected between the experimental group and the control group regarding to bodyweight loss, food intake, water intake, fURI score and adoption rates.

Results suggest offering hiding enrichment could help coping with the new environment by reducing physiological stress. Although, inter-animal variability is high and hiding boxes may not reduce stress in all cats, at least the option of hiding and perching should be offered. No significant differences were registered between the experimental group and the control group regarding to bodyweight loss, food intake, water intake, fURI score and adoption rates but descriptive statistics suggests the importance of a hiding box on improving adaptation, reflected by less mean decreases in bodyweights for cats in the experimental group. Determining body weights could therefore act as a relatively easy and practical way for recognizing impaired welfare. This study highlights the importance of hiding enrichment and applying this in practice will be an important step in increasing welfare in shelter cats.

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INTRODUCTION

Domestic cats are one of the most popular pets worldwide with early evidence for close human-cat relationships and domestication dating to ca. 5300 years ago (66). Nowadays, the total cat population in the Netherlands consists of circa 2,6 million individuals of which annually, approximately 52.000 cats end up at facilities for shelter and reassignments due to all different kinds of reasons for relinquishment (22, 67). These sudden change of environment and introduction of unfamiliar routines, persons and animals asks for adaptive mechanisms. Cat's needs could be inadequately met for this new, confined situation will not allow them to perform behavior to which it is inherently adapted to (45, 49). Failure to cope with these environmental changes can indicate a decline in welfare (5). When an individual perceives a challenge to its homeostasis, this evokes a biological response, which can be defined as "stress". The threat is called the "stressor" resulting in series of physiological events increasing autonomic activity and metabolic changes, initiating the "fight or flight" response (36, 37). Exposure to acute, short-termed stressors are associated with the hormones norepinephrine and epinephrine, hence preparing the cat for the fight and flight response by increasing heartbeat, respiration rate, glucose metabolism and glucocorticoids (GCCs) (37). When animals are exposed to repetitive chronic and long-term stressors, suppression of both cellular and humoral immune system occurs, which may lead to immunodeficiency and making the cat more vulnerable for all kinds of infection. When prolonged high levels of GCCs persist, this will eventually cause an inability of the feline body to adapt, resulting in a reduction of the activation of the hypothalamic-pituitary-adrenal axis response to acute stress and causing suppressed immune responses and reduced body weight (6, 22, 37).

Previous studies indicate feline upper respiratory tract infections (fURI) are mainly caused by feline herpesvirus (FHV) and feline calicivirus (FCV) and is known for being the most commonly encountered infectious disease complex in animal shelters. It impairs a cat's welfare by affecting its physical well-being and is hard to eliminate from shelter situations (2,4,63). Although, in the study of Selman et al. signs of fURI were not observed, other studies did indicate high prevalence of fURI in shelter cats (2,11,54,63). The absence of signs in the study of Selman et. al. might be explained by the use of a less discriminating scoring system and/or can be a (in)direct consequence of the hiding box in quarantine situations, since this may have helped to reduce stress in these cats and thereby lowering the change of reactivation of latent infections (2,4,54,63). The low prevalence of signs of fURI in the previous study can also be explained due to an observation period that might have been too short to identify infected cats since recrudescence of FHV infection after a trigger due to exposure to stressors in carrier cats can take up to 11 days (63). In the current study we scored signs of fURI over a period of 14 days, using a different scoring method.

Besides these physiological changes, behavioral changes could also be observed. Chronic stress may lead to increased behavioral inhibition, increased hiding behavior, inhibition of elimination of both feces and urine and decreased food intake or even anorexia (6,37,39,59). Monitoring body weight can serve as a useful and feasible parameter to detect consequences of stress and indications of impaired welfare in shelter cats. Among others, loss of body weight can be the result of the catabolic effects of cortisol and catecholamines or a decreased intake of food, which both can be triggered by stress and has been observed by various studies before (25,57,58,59). Selman et al. detected a mean weight loss of 6,34% in the hiding box group and a mean weight loss of 7,71% in the control group over 12 days (54). In Tanaka et al. weight loss was followed during 21 days in 60 individually housed cats. 34 (57%) of those cats lost $\geq 5\%$ of their body weight while being in the shelter (63), indicating weight loss in shelter situation is a serious concern.

Measuring stress is difficult, because – apart from the inter-animal variability in stress response- the actual data collection in practical circumstances can be experienced as stressful in animals and could therefore affect the results (36). In the current study we aimed to determine an easy measurable and practicable parameter for monitoring stress in shelter cats which can be used by shelter workers and volunteers with the purpose to recognize, reduce and even prevent stress, thereby optimizing health and welfare in shelter cats. Hiding enrichment can be an important part of improving welfare since cats than will have an opportunity to remove themselves from stressors. When behavioral options become limited by confinement, the cat could be unable to avoid the stressor (36). As previously published, cats prefer any type of enrichment that offers hiding opportunity when confined to a small area like a cage. Also, cats prefer to monitor their surroundings from higher levels, so elevated points like perching shelves and raised platforms are favored above ground level (20,33,48,49,54,68). Offering that possibility, may affect their welfare in a positive way.

In the previous studies of Vinke et al. and Selman et al. the Cat Stress Score (CSS) - developed by Kessler and Turner - was used to determine the effect of hiding enrichment on the amount of stress in newly arrived shelter cats (31,54,68). Results presented overall higher CSS in cats without access to hiding enrichment in a caged environment (54,68). Although, CSS is a non-invasive method of scoring by which reliable results have been achieved, it also requires knowledge, experience and time (31,63). Due to these reasons, this method will be less applicable for shelter employees in practice and in this present study we will look at place preference locations in order to determine behavioral needs in captive areas. The pilot study of Vinke et al. showed that cats in the “hiding box group” spent most of the observed time (55%) in the hiding box whereas the cats in the control group (no hiding box) spent most of the total observed time behind the litterbox (45%) (68). Hiding behind the litterbox points at some kind of hiding behavior which has to be satisfied in a different way by lack of opportunity to hide in a proper hiding enrichment and may indicate an impaired welfare (51). In the successive study of Selman et al. similar results have been found in the “hiding box” group, indicating a possible relation between the presence of hiding enrichment and stress (54,68).

In order to improve welfare as much as possible for cats placed in temporary housing, ensuring appropriate housing conditions is essential. Several studies were conducted with the aim of examining if cat-cat contact has a positive effect on stress reduction and therefore can improve the adoption chances. (30,31,42,56). Ottaway et al. hypothesized that group instability and the presence of unrelated adult cats in social housing situations in shelters creates more stress than single housed cats and found significantly higher mean Cat Stress Scores in the cats in social housing compared to those in individual housing (42). Similarly, cats that are poorly socialized to other cats typically experience stress when group housed (30), and all cats experience some stress when new cats are introduced to the pen (56). Another study compared three “treatments”: basic single housing, enriched single housing (both individual housing situations) and basic communal housing (group housing). They found a basic single treatment had the lowest percentage adopted cats in 21 days and higher stress scores than the other treatments. The three alternative treatments did not differ significantly on any measure (20). Smith et al. 1992 studied group-housed cat and concluded social contact between the – at first- unfamiliar cats had a negative effect on stress levels in the first period but helps reduce stress levels during longer stays (months)(56). Since results from previous studies are inconclusive, further research will be necessary to evaluate the effect of social housing on stress levels in shelter cats.

The amount of time cats spend in close proximity can be an indication of affiliation and may be correlated to a decline or increase in stress levels. The opportunity to maintain a distance of 1-3 meters distance between themselves and other cats is mentioned in several studies (3,31). Various previous studies

identified temperament dimensions in domesticated felines and although these studies have not used standardized methods, quite similar results were seen (17,24,35). One of these dimensions is “Cat-Cat Sociability”, which can be associated with an increased amount of anxiety or stress, when a cat is housed in a multi-cat household (like units in a rescue shelter) (24). A way of examining contact between unrelated conspecifics, is by introducing the “Social Adaptation Score”, a new method of determining the amount of time a focal animal spent in the presence of conspecifics during the first week of social housing in a rescue shelter. We will refer to this “Social Adaptation Score” as “SAS” in the remainder of the text.

Social cat to cat behavior plays a large role in chance of adoption by new owners, as well as a variety of other factors. Several studies have examined reasons for pet selection in rescue shelters and showed that appearance, social behavior with potential adopter and personality were the main reasons for adoption (20,44,69). However, in practical situation, the short amount of time available for selection in shelter situation, makes it harder to collect details regarding cats personality and temperament. Results of Dybdall et al. and Weiss et al. suggesting that approaching and social greeting behavior may increase chance for adoption (13,69). Cats with lower stress scores showed more positive approaching behavior toward potential adopters and therefore were more likely to be adopted sooner (33). In addition, it is shown that cats without a possibility to hide, had the lowest adoption rates (20). According to different housing situations, several studies did not show any significant differences between adoption rates in socially housed cats compared to individually housed cats (19,21,54).

The aim of this study was to give insight into the effects of a hiding box on stress levels in shelter cats by determining locations for place preference in confined areas, body weights and the prevalence of clinical signs of fURI in newly arrived cats during the first 14 days in individual housing in the quarantine area and the first 7 days in consecutive social housing in a Dutch animal shelter. Based on the literature, we expect that cats with a hiding box will show the least decrease in body weight and represent the lowest fURI scores and recover more quickly during their stay in the quarantine area, reflected by paying less visits to the hiding box as controlled by place preference (54,68).

For the social housing conditions we expect that cats who spent their time in close proximity to other cats will show a decrease in stress levels reflected by the least decrease/most increase in body weight, the lowest prevalence of clinical signs of fURI and/or spent the least amount of time in concealed types of hiding enrichment and/or on elevated levels, measured by scoring place preference in social housing units. Based on results from previous studies we expect that measuring bodyweight losses in shelter cats is a reliable and applicable method for shelter staff members to act as an indicator for stress (54,63,68).

At length, we expect that cats originating from the experimental group (the “hiding box” group) in quarantine housing spent less days in the shelter than cats deriving from the control group, which will be reflected by higher adoption rates for experimental group cats.

MATERIALS & METHODS

Study site

The collected data were obtained at “Stichting Sticht Asyl voor Dieren”, a Dutch animal shelter facility that offers shelter and rehomes dogs and cats in Utrecht and its surroundings with a maximal capacity of housing 225 cats at once. In 2016 they housed a total of 616 cats. 521 were brought in as a stray, 82 were relinquished animals and 13 were born in the shelter (65).

Subjects

Cats that were included in this present study, were selected based on the following criteria:

- Breed: merely European domestic short hair cats or “house cats”.
- Gender: both male and female cats were included.
- Age: range between 1 and 11 years of age.
- Health status: the cats that were included in this study showed no signs of illness or injury. A general physical health exam was performed at intake.
- Surrender type: included cats had either been captured as strays or had been relinquished/surrendered by previous owners.
- Sociability: All cats were suspected as adoptable and feral cats were not included.

These inclusion criteria were conforming to the studies of Vinke et al. and Selman et al. (54,68).

The group size depended on the rate of intake at onset of this study and was conducted during May and June 2017. Eventually, 16 cats met the requirements as mentioned above. To avoid any selection bias, all of the selected cats were randomly assigned into two research groups; the experimental group with a total of 9 animals (6 males, 3 females) were offered a hiding box and the control group which consisted of 7 animals (5 males, 2 females), did not have access to a hiding box. Ages ranging between 12 and 133 months in the experimental group, with a mean of 63.00 months ($SD = 43.24$) and median of 66 months. The ages of cats in the control group ranged between 24 and 93 months, with a mean of 58.86 ($SD = 25.50$) and median of 60.00 months. Since 10 out of 16 cats were strays, ages were estimated by the shelter's vet using teeth and total body condition and thus may deviate from their biological ages. The other 6 cats were relinquished and came with a passport which contained their dates of birth.

Housing conditions

All newly arrived cats remained in individual housing in the quarantine area for 14 days, as established by Dutch law (1). The shelter possessed 5 quarantine areas, each containing nine separate cages (fig. 1). Cages consisted of solid plates of stainless steel (L x W x H: 70 x 70 x 60 cm) with the perching shelf (L x W: 70 x 30 cm) at a height of 30 cm. Cages contained steel bars at the front and visual contact between cats was not possible. Each quarantine cage contained an open litterbox, a food- and water bowl and a perching shelf. Since cats prefer materials that maintain a constant temperature, towels were placed covering the entire surface of the cage, preventing place preference due to motivation for a certain substrate (48). Towels were placed conforming to Vinke et al. and Selman et al; one on the perching shelf, two or three covering the bottom of the cage and one in the hiding box (in the experimental group) (54,68). Each cat of the experimental group had access to a hiding box, that was placed to the back in the right corner of the cage, beneath the shelf.

Agreements were made with the shelter staff, that newly arrived cats who met the inclusion criteria of this study, were merely placed in quarantine A, since the position of cameras and cables were most optimal in this room. Only 1 cat had to be placed in quarantine C because of lack of space.

Daylight was available through one window and seconded by a fluorescent lamp between circa 8:00h and 16:30h. Ambient temperatures could be regulated manually by opening the window or regulating the radiator.

After the legally determined two-week period in quarantine area, the cats were available for adoption and therefore moved to either a unit with other cats (social/group housing), or individual cages (individual housing). This decision was conducted by educated guesses of experienced shelter staff and depended on health status and/or temperament of the cat and its ability to tolerate conspecifics. In group housing, cats were placed in one of the four available units (approximately 12 square meters) with variables numbers of conspecifics (maximum of 15 cats per unit). Each unit provided unlimited access to an outdoor fenced area (6-7 square meters) and each units contained 3 or 4 open litterboxes, multiple food- and water bowls, several commercial cat toys, open resting baskets with towels, cat igloos, hammocks, a multi-level cat tree, chairs and 4-5 shelves. Individual housing could be compared with the quarantine situation, only cages were places against the wall in a unit.

Standard procedures

Presence of microchips were checked in each newly arrived cat upon intake. Each Tuesday and Friday, the shelters veterinarian was present for providing health checks, vaccinating, flea- and tick treatments and microchipping if necessary. After 10-14 days, tomcats were castrated in the shelter and female cats were taken to the clinic on Tuesday and usually returned next Friday.

Cleaning of the cages and units and caretaking of the cats was carried out daily at set times by one of the four shelter staff members. Daily routine started at 8:30h and consisted of swiping the bottom of the cages, emptying and refilling the litter boxes (Linda Hout kattenbakvulling®), refreshing drinking water and serving food. Cats remained in their cages during cleaning of the cage and towels were replaced when necessary. After the two-week quarantine period, cages were thoroughly cleaned and disinfected before reusing.

Experimental setup

Data collection was conducted between 10:00h and 15:30h, for this was between the morning and afternoon feeding- and cleaning routines and interaction with staff members was minimal. During the behavioral assessments the cats were observed using a video camera (Bascom® DVR). Influence and any kind of interaction of the observer with the cats was minimized as much as possible by locating the observer and its equipment in an office space, out of sight, smell and sound of the observed subjects. The

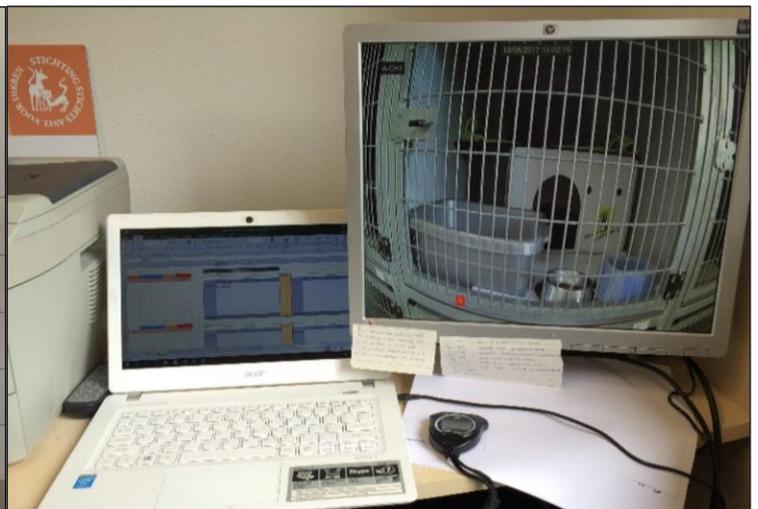


Fig. 1. The nine separate quarantine cages in quarantine area A Fig. 2. Workplace set-up for observer

observer scored the behavioral parameters through a computer screen connected with the cameras and used a laptop for entering data (fig. 2). In the quarantine area, one camera per cat was placed in front of the cage so a total overview of the entire cage was visible on the screen (fig. 3). After placement of the camera, an adjustment period of 2 minutes was maintained before recording behavioral data. fURI scoring took place after collecting behavioral data, to minimize influence of social contact between observer and cat prior to the behavioral scoring. All data were scored by the same observer.

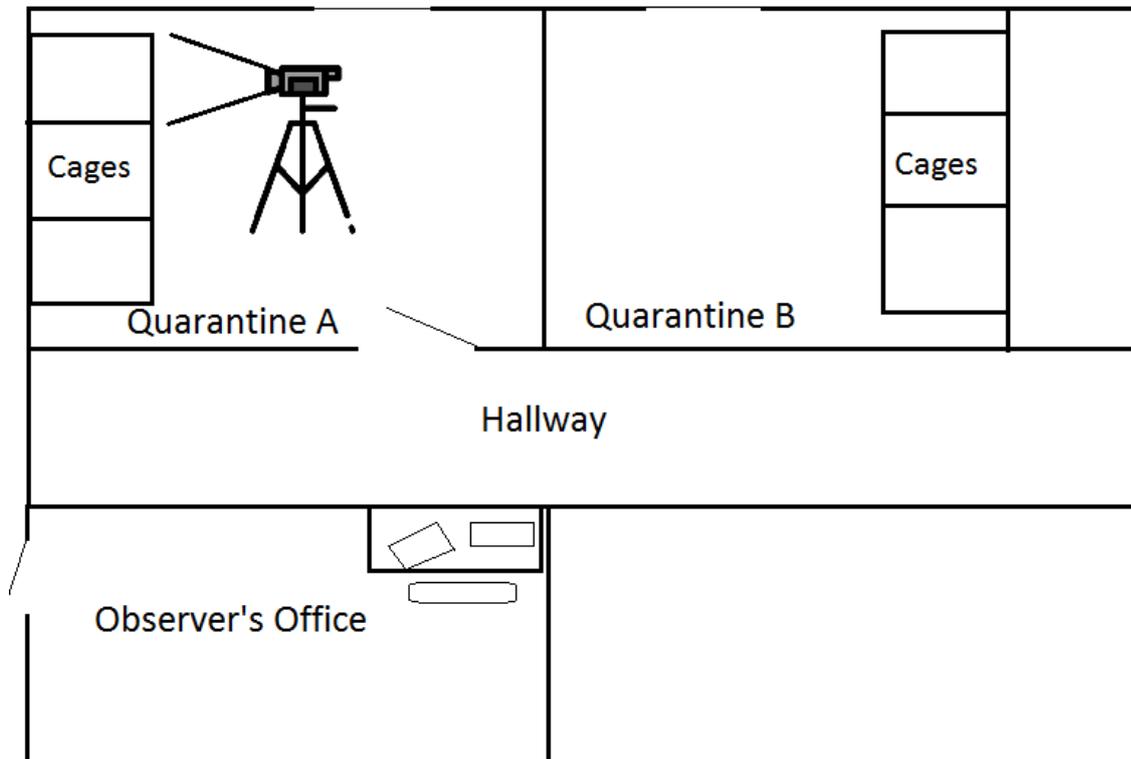


Fig. 3. Experimental set-up in quarantine area and location of the observer in an office space, separated by a hallway

In social housing, two cameras were placed against the wall in the unit; one indoors and one outdoors (fig. 4-5). Unfortunately, some blind spots could not be avoided, although losing sight of a recorded cat was compensated by extending total time of recording. Ambient temperatures were measured during each single behavioral observation by means of a climate measuring instrument placed on the table in the quarantine area or in the indoor area of the unit. Temperatures varied between 19.3 °C and 25.9 °C with a mean of 22.04 °C (SD = 0.25) in the experimental group and 22.29 °C (SD = 0.37) in the control group.



Fig. 4. Camera overview in social unit - indoors



Fig. 5. Camera overview in social unit - outdoors

Hiding box

The hiding boxes used in this study, derived from the same batch as used in the previous studies from Selman et al. and were provided by the Dutch Society for the Protection of Animals. Each hiding box consisted of cardboard and measured L x W x H: 44 x 31 x 26 cm (fig. 6). For it had only one entrance in the front, a second entrance was manually made left of the first one, to mimic conditions used in previous studies of Vinke et al. and Selman et al. (54,68). Boxes were not reused between cats and replaced with new boxes when being contaminated with feces and/or urine.



Fig. 6. The hiding box

Data collection

Data were collected between May 12 and July 10, 2017.

Scoring behavioral parameters

Place preference in quarantine

Place preference was scored on days 1,2,3,5,7,9,12 and 14 in the quarantine area in order to get more insight in the possible effects of a hiding box on preferences for a specific place in their cage. Since the most accurate way to measure behaviors – both frequencies and durations- is through continuous recording, an all occurrences sampling method had been used (34). The total observation time per cat was 20 minutes once a day, measured on the established days as mentioned above.

Following situations were distinguished:

- Being on the perching shelf
- Being in the hiding box (only for the experimental group)
- Being elsewhere-hiding
 - a. being in the litter box
 - b. being behind the litter box
- Being elsewhere- non hiding

“Being elsewhere-hiding” was divided into two subdivisions, since lying in or behind the litterbox could be interpreted as alternative hiding behavior (6). Cats met the criteria of a certain situation when “the cat had more than two paws or more than 50% of its total body weight in or on that part of the cage” (54,68).

Time spent in a certain situation was registered and expressed as a percentage of a total of 20 minutes to determine a daily place preference per animal. These fractions were averaged per research group to obtain mean daily scores. Subsequently, the mean place preference score of the total observation period was determined per research group. The purpose for this differentiation was to detect if alternative hiding activity could be seen in absence of proper hiding opportunities, as mentioned in several previous studies (6,20,33).

Place preference in social housing

In order to relate space use in a confined area (adoption room/unit) to stress levels, scoring place preference in adoption units happened on day 15,17,19 and 21 with an all occurrence sampling method and a daily recording time of 30 minutes

Following situations were distinguished in case of group housing (47):

1. Being on ground level
 - G-ca: being in the central area
 - G-pa: being in the peripheral area
 - G-hp: being in any type of enrichment with hiding purpose
2. Being on high level (perching shelves, on top of a chair etc.)
 - H-ca: being in the central area
 - H-pa: being in the peripheral area
 - H-hp: being in any type of enrichment with hiding purpose

For the cats will also have unlimited access to an enclosed outdoor area, a combination with following situations has been included:

- A. Being indoors
- B. Being outdoors

Cats will be allocated to “being in the peripheral area” by determining cat’s distance to the wall by means of one floor tile (60x60 cm). Cats met the criteria of situation 1 or 2 and A or B when “the animal had more than two paws or more than 50% of its total body weight in or on a specific area ,marked as in figure 1, or in any type of enrichment”. During the observation period, the time spent in each situation was registered and expressed as a fraction of a total of 30 minutes to generate a mean daily place preference per cat. The mean daily scores of all cats on the 4 observation days were averaged to get a mean place preference for the total observation period. Mean daily percentages of hiding behavior (both indoors and outdoors, both high level and ground level) were calculated for each observation day and for the overall period in order to get more insight in hiding behavior of cats in social housing situations.

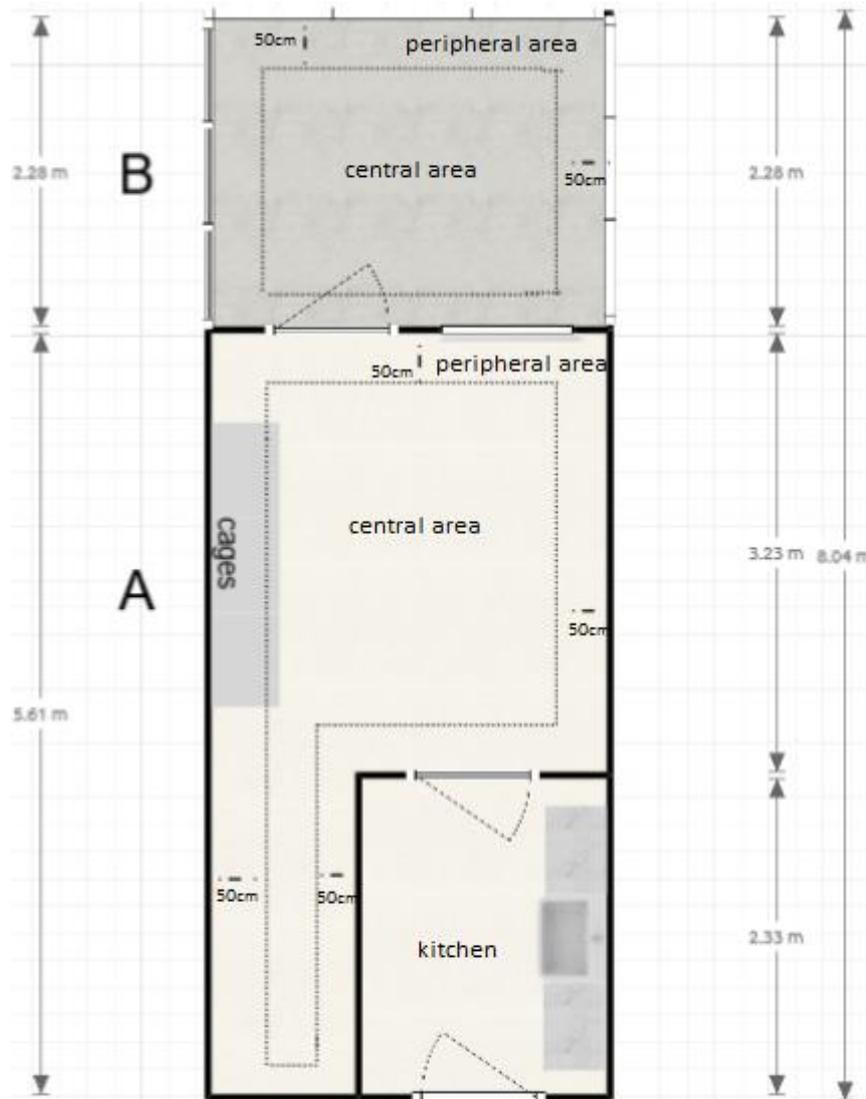


Fig. 7. Place preference locations in unit

Social adaptation scores

In group housing, cats are forced to live in close proximity of each other but many cats are not adapted to this social constructions (48,49). In this study the “social adaptation score” (SAS) was introduced in order to evaluate a possible relationship between cat-to-cat distance and stress levels. SAS was scored on observation day 15,17,19 and 21 in social housing units with the all occurrence sampling method. This parameter described the amount of time a focal animal spent in close proximity to a conspecific, expressed as a percentage of the total observed time of 30 minutes for each observation day per cat. Added to that, following criteria were maintained:

- When distance between focal animal and other cat > 0,6 meter, this will be classified as “in proximity” and this cat will be given a SAS of 1.

- When distance between focal animal and other cat < 0,6 meter, this will be classified as “not in proximity” and this cat will be given a SAS of 0.

In previous studies, a length of 1 meter between cats is being used often as a measure of proximity (9,10). In the present study a closer proximity of 60 centimeter will be used to define proximity between conspecifics because - given the size of the unit - cat-to-cat contact will often happen within a range of 1 meter, even if unintended. In Suchak et al 2016, body length was used as a measure but because body lengths are difficult to determine by means of video imaging, floor tiles were used as a measure of proximity (62). Floor tiles in group units measured 30x30 cm, and could be served as a tool to observe cat-to-cat proximity in a more reliable way. Floor tiles were simulated in outdoor area by measuring 30x30 cm areas and marking them with tape. Visualization of tiles was accurate through cameras.

Scoring physical parameters

fURI

The absence of fURI in Selman et al. could be caused by an inadequate scoring method (54). In the current study, the scoring method of Dinnage et. al. was used in which cats with any clinical signs related to upper respiratory tract infections will be identified as being infected (11). The scoring method of Dinnage et al. is defined as follow; “cats with ocular or nasal discharge, sneezing with or without nasal congestion, coughing, dyspnea, or blepharospasm (in conjunction with other signs) were considered to have an upper respiratory tract infection”. Per cat, each sign was rated a 0,1,2 or 3 and scores were accumulated to define severity (fig. 8)(11). Signs of fURI were scored on day 1,2,3,5,7,9,12 and 14. Sneezing and/or coughing could be visualized at screen during the 20 minutes of observing, the other signs were scored per cat directly after the recording period. Scores were added up per cat and mean fURI scores were determined for the quarantine period of 14 days for both treatment groups.

Clinical signs	Score 0	Score 1	Score 2	Score 3
Ocular discharge	Non	Small amount of serous discharge	Large amount of serous discharge	Mucopurulent discharge
Nasal discharge	Non	Small amount of serous discharge	Large amount of serous discharge	Mucopurulent discharge
Respiration	Normal	Mild difficulty breathing (mildly increased chest movements with no regular abdominal movements present during breathing)	Moderate difficulty breathing (increased chest movements with some regular abdominal movements present during breathing)	Severe difficulty breathing (increased chest movements present during breathing)
Sneezing	Non	Sneeze 1 time/20 min	Sneezes 2-3 times/20 min.	Sneezes 4 or more times/20 min.
Coughing	Non	Coughs 1 time/20 min	Coughs 2-3 times/20 min.	Coughs 4 or more times/20 min.
Demeanor	Bright, alert, reactive	Quiet, lethargic	Depressed but responds to human contact	Severely depressed, demeanor does not change in response to human contact

Fig. 8. Scoring method of Dinnage et al. for scoring fURI

Body weight

Cat’s body weights were obtained using the FCE, an electronic scale with a maximum of 30 kg and an accuracy of 10 g, on day 1 (week 0). The subsequent stay in shelter was then divided into three periods: week 1 (days 2 through 7), week 2 (days 8 through 14) and for cats that stayed long enough, a third period was added: week 3 (days 15 through 21). Body weights were measured by the same observer on day 1, 7, 14 and 21 and always after scoring the behavioral parameters to avoid influence of animal handling on behavioral data outcome. Each body weight was registered and an daily average was calculated per

research group. Weight loss and weight gains were registered per cat as a percentage of the total body weight compared to week 0 and between the weeks. All weight losses and gains were averaged per research group.

Food- and water

Cats were served Adult Royal Canin® SC365D dry cat food twice a day. Amounts that were given were based on their body weight at intake and calculated by following formula (40):

RER (resting energy requirements) = Bodyweight in kg^{0,75} x 70 kcal.

DER (daily maintenance energy requirements) = 1,2 x RER

Royal Canin® SC365D dry cat food grams/day = DER/4,066kcal/gram

4,066 are in kcal/gram, because metabolized energy is in kcal/kg and NCR2006 for Royal Canin® SC365D dry cat food is 4066 kcal/kg. Shelter cats in quarantine cages have limited space for moving around, therefore the DER of 1,2 (inactive neutered adult) was chosen. In the previous study cats were offered a standard gift of 50 g/d Royal Canin SC365D dry cat food which appeared to be inadequate for cats over 4 kg to meet their daily nutritional needs (54). In this study, body weight loss due to offering inadequate amounts of calorie requirements were excluded.

On daily base, 300 milliliters of water was offered and remaining water was measured each morning before refreshing. Through the day, water bowls were not being refilled, except when bowls were pushed over or excessive spilling happened.

Elimination of urine and feces was registered for each individual cat at both morning- and afternoon rounds on observation days 1 through 13.

Adoption Rates

Adoption rates were registered in order to estimate the effect of a hiding box in quarantine on adoption numbers and rates by determining the number of days between leaving the quarantine cage and day of adoption. Mean adoption rates were determined for each treatment group and total adopted population was studied for outliers.

Statistical analyses

For this study, a randomized controlled trial (RCT) design was used. Microsoft Excel 2016 (Microsoft Corp, Redmond, Wash) was used to maintain data and generate descriptive statistics. Statistical analyses was done by means of IBM SPSS Statistics 25 (IBM Corp, Amronk, NY).

To examine normal distributions, Shapiro-Wilk tests were used due to evidence for providing the highest power in studies with small samples sizes ($N < 30$) (46). A value of $p < 0.05$ was being considered as statistically significant. When the assumption of normal distribution was not met, non-parametric test were used. Mann-Whitney U tests were performed for mean place preference frequencies for each location between treatment groups. Descriptive analysis was used to examine potential relations between place preference, time and research groups. Because sample sizes reduced to only 5 cats (experimental group: $N = 4$, control group: $N = 1$) in social housing situation, descriptive analysis was used.

A Shapiro-Wilk's test ($p > 0.05$) and visual inspection of the histogram, normal Q-Q plots and box plots indicated that the examined values for body weights at day 1, 7 and 14 were not normally distributed. (Shapiro et al.). Differences were compared within groups over time points (within subject factor) and between groups (between subjects factor). A linear mixed model was carried out with "cat" as random effect and group, time, gender, age and surrender type ('stray' or 'relinquished') and their interactions as

fixed effects to investigate differences between the two groups. Optimal structures were determined for fixed factors by choosing the lowest AIC (Akaike’s Information Criterion) and degrees of freedom. Significant effects were included in rebuilding the best model.

Mann-Whitney U tests were performed to examine if the two treatment groups differed significantly from each other regarding to mean fURI scores per cat, overall mean fURI scores for both treatment groups and mean adoption rates. Spearman’s rho correlation test was used for detection of significant correlations between fURI scores and treatment groups and surrender type. Differences in fURI scores between treatment group and observation days were investigated by performing a Friedman’s ANOVA.

The endpoint of interest in this study were set by adoption rates. A Cox proportional hazards model was used to quantify relationships between research group and total length of stay in shelter (8). Cat 17 was censored for being euthanized at day 14. The non-parametric Spearman’s R Correlation Test was performed to investigate correlation between adoption rates and ages in both groups. Correlations were performed two-tailed with a $p < 0.05$.

RESULTS

Place preference

At first, a Shapiro-Wilk test was conducted to investigate each mean place preference location per cat of the quarantine period for normal distribution for both experimental as control group. In the experimental group, normality was found for “hiding box” ($D(9) = 0.899, p = 0.244$) and “being elsewhere” ($D(9) = 0.914, p = 0.346$). For control group, normal distributions were found for “hiding behind litterbox” ($D(7) = 0.811, p = 0.053$) and “being elsewhere” ($D(7) = 0.936, p = 0.602$). Non normal distributions for control group were found for “perching shelf” ($D(7) = 0.674, p < 0.01$) and in experimental group for “perching shelf” ($D(9) = 0.810, p < 0.01$) and “behind litterbox” ($D(9) = 0.552, p < 0.01$).

During the entire observation period, no cats were observed sitting in the litterbox. Medians of 0.0% were found for “perching shelf” ($IQR = 0.04$) and “behind litterbox” ($IQR = 42.5$) in the control group and for

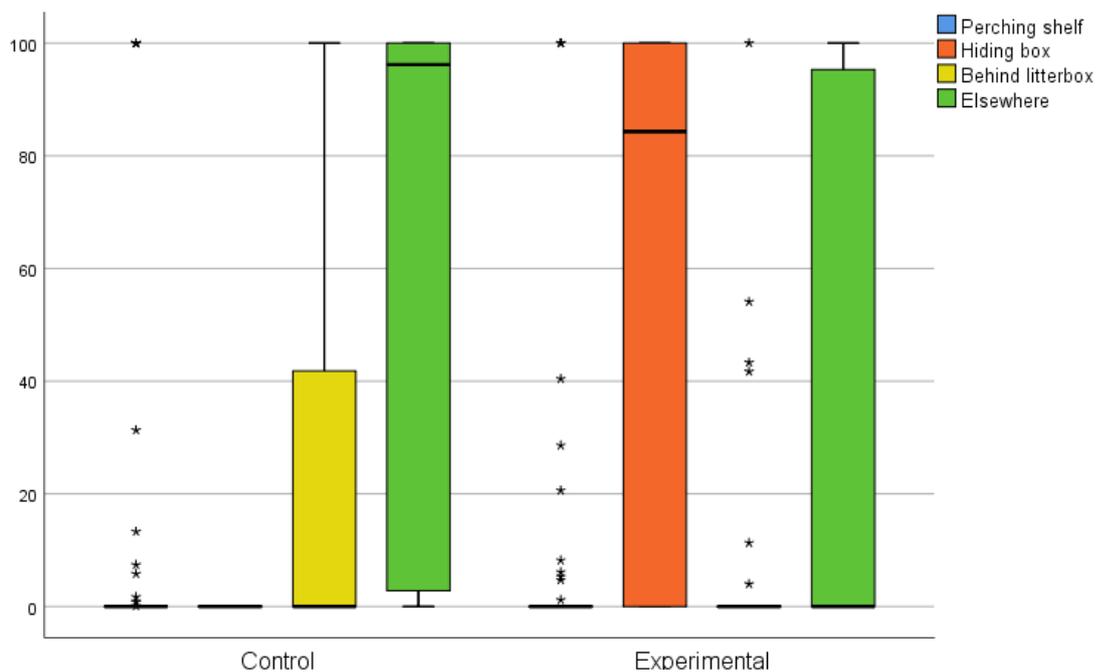


Fig. 9. Medians ($\pm IQR$) for each place preference location displayed for both experimental ($N = 9$) and control ($N = 7$) group for the entire observation period of 160 minutes

“perching shelf” ($IQR = 0.0$), “behind litterbox” ($IQR = 0.0$) and “being elsewhere” ($IQR = 95,7$) in the experimental group. In the control group, a median of 96.2% ($IQR = 97.9$) was found for “being elsewhere” and for the experimental group a median of 84.3% ($IQR = 100.0$) was found for “hiding box”(fig. 9).

Figures 10 and 11 were studied for daily medians ($\pm IQR$) in order to study daily variation for each place preference location in both treatment groups.

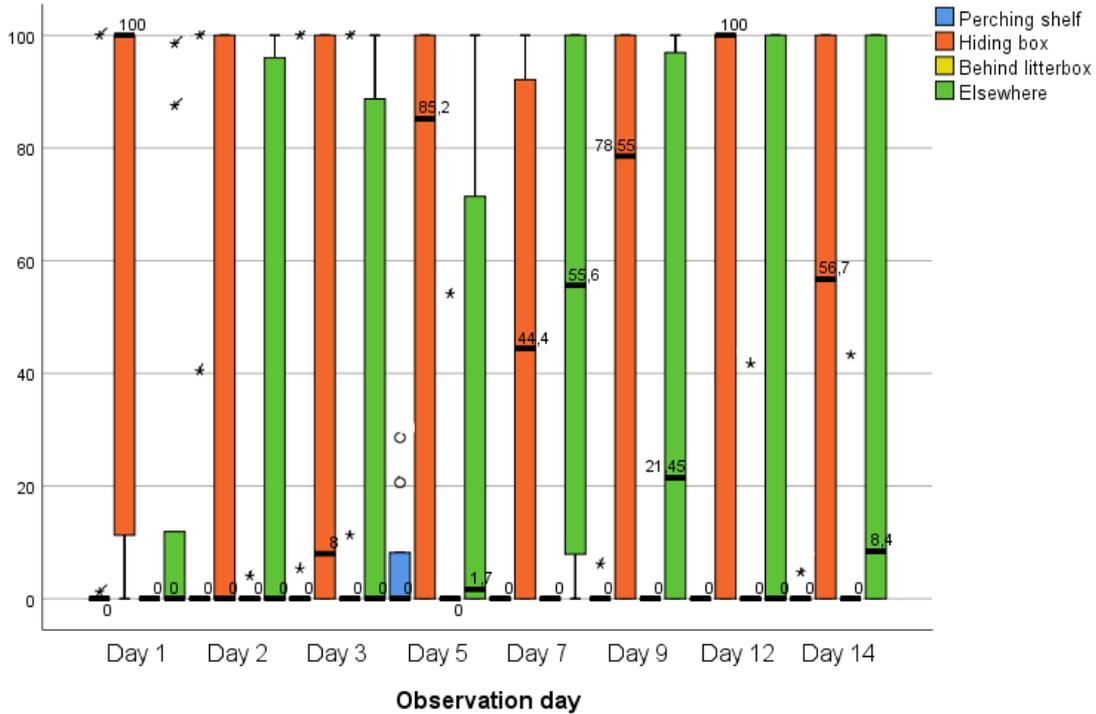


Fig. 10. Clustered boxplot with daily medians ($\pm IQR$) for each place preference location in the *experimental* group (N = 9).

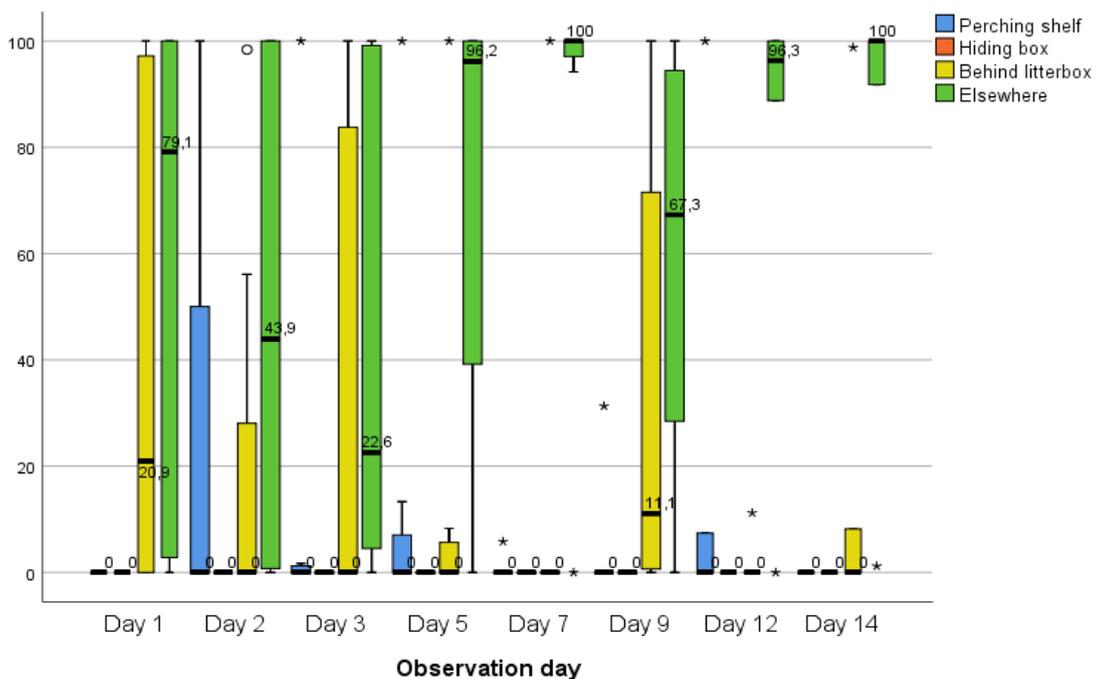


Fig. 11. Clustered boxplot with daily medians ($\pm IQR$) for each place preference location for the *control* group (N = 7).

Cats in the control group spent 67% of their time elsewhere versus 38% in the control group (fig. 12). Mann-Whitney U test indicated that cats in the control group spent significantly more time “being elsewhere” ($U = 12.00, z = -2.067, p = 0.039, N_{1,2} = 9,7$) compared to cats from the experimental group. Cats from the experimental group spent most of their time (53%) in the hiding box. A dissimilarity between the two groups was seen in time spent behind the litterbox: 24% versus 3% in respectively control- and experimental group. 6% of the experimental group spent their time on the perching shelf, whereas control group cats spent almost twice as much time (10%) on this elevated level. Mann-Whitney U tests showed no significant differences for “perching shelf” ($U = 31.50, p > 0.01$) and “behind litterbox” (experimental group: 3%; control group: 22%; $U = 18.50, p > 0.01$) between the two treatment groups.

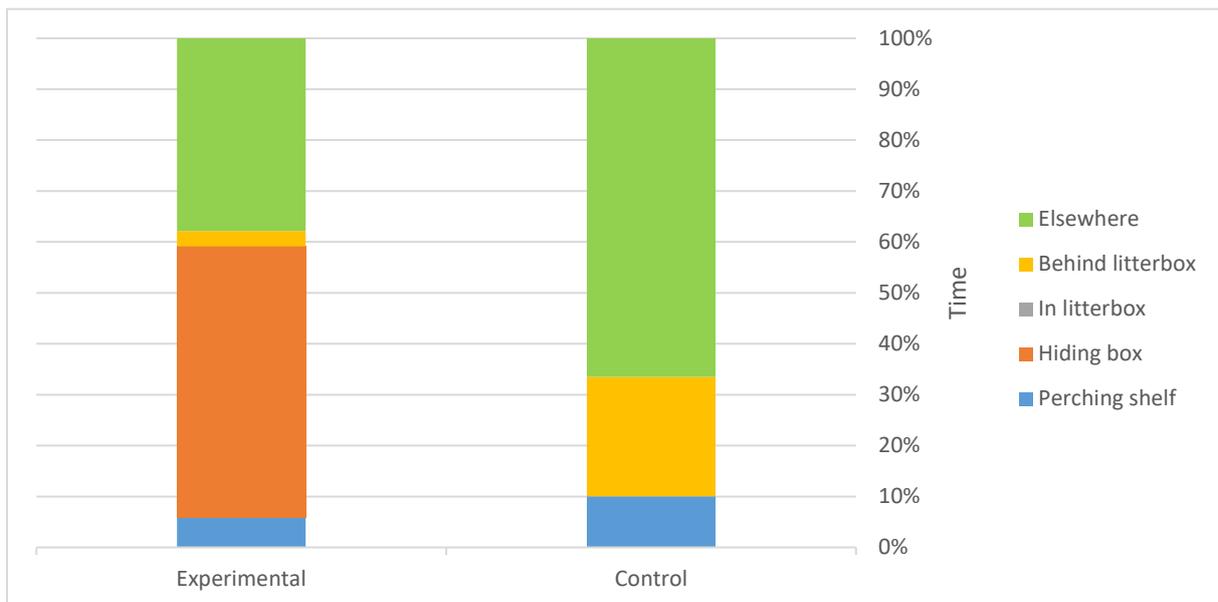


Fig. 12. Mean percentages of time spent in each place preference location in quarantine for the total observation period of 160 minutes, visualized for both the experimental group ($N = 9$) and control group ($N = 7$).

In the experimental group, a decline was noticed in time spent on the perching shelf in the first 2 observation days (respectively 11% and 16%) versus time spent on the shelf in the last two observation days (respectively 0% and 0.5%) (fig. 13). The highest percentage for place preference for the hiding box was found at day 1 (67%), however this decline was not continuously during the following observation days. Place preference for being behind the litterbox in the experimental group was seen on 4 out of 8

observation days (day 2,3,5 and 14). In the control group, each single observation day (1,2,3,5,7,9,12 and 14) cats spent time behind the litter box.(fig.14).

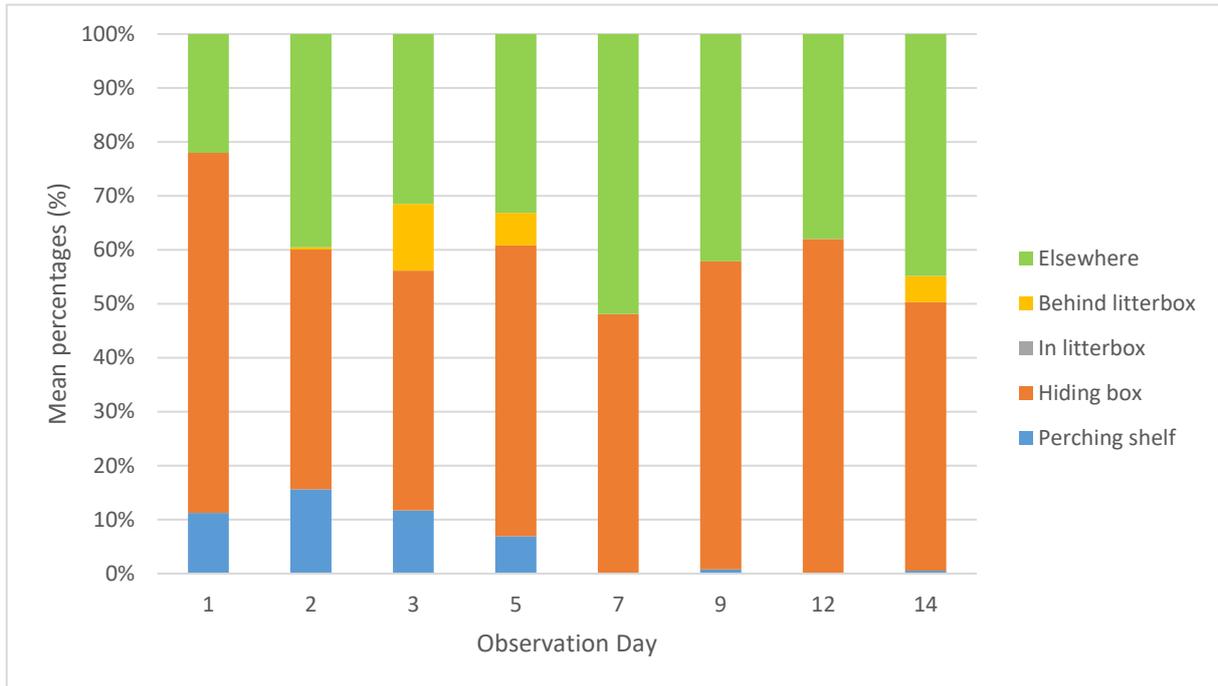


Fig. 13. Mean frequencies of place preference in the *experimental* group (N = 9) at day 1,2,3,5,7,9,12 and 14, expressed as a percentage of the daily observation time of 20 minutes.

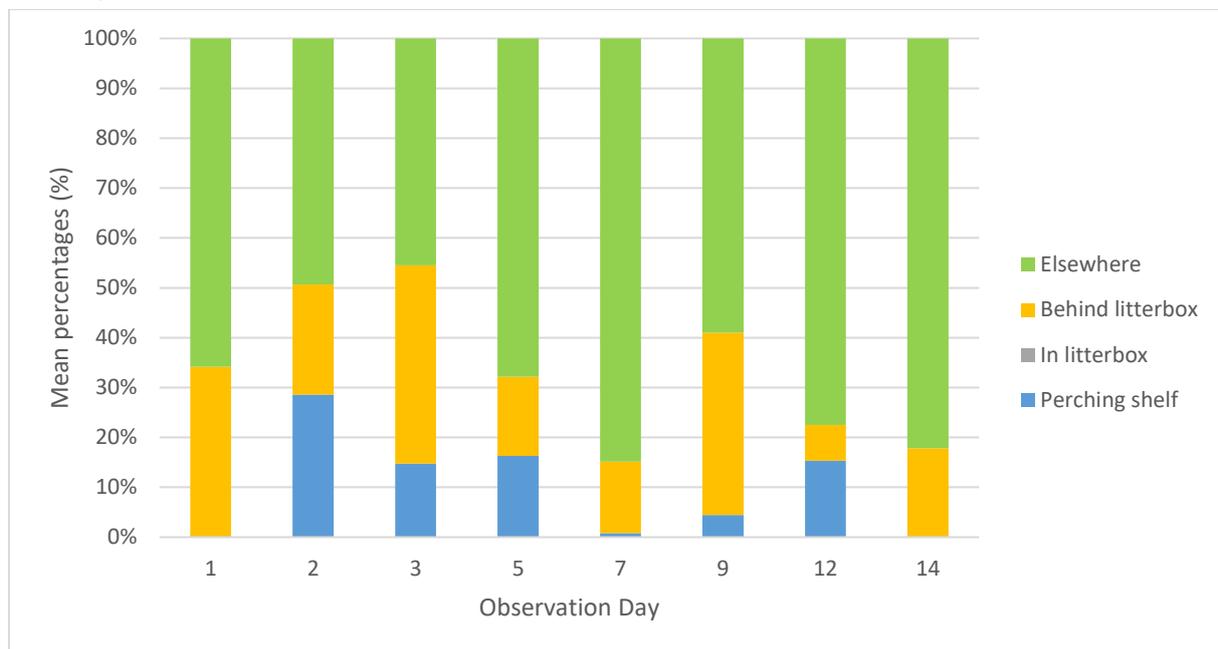


Fig. 14. Mean frequencies of place preference in the *control* group (N = 7) at day 1,2,3,5,7,9,12 and 14, expressed as a percentage of the daily observation time of 20 minutes.

Because inter-animal variations were high and motivation for certain place preference locations seemed to differ, fig. 15 and 16 were studied for mean individual place preference locations. In experimental group, cat 4,14,17,18 and 22 showed a clear preference for the hiding box, while cat 1, 7, 11 and 16 had an obvious predilection for “being elsewhere”. Cat 11 only spent 0.2% in the hiding box versus cat 18, which spent 100% inside the box.

In the control group, cat 13 tried to hide beneath the towels located at the bottom of the cage which resulted in high scores for being “behind the litterbox” for it could be interpreted as alternative hiding behavior. All the other cats in control group spent most of their time “elsewhere”. Cat 9 and 15 spent all of their time elsewhere. When compared to the mean for being on the perching shelf, cat 19 showed a clear preference and contributed to the mean of 9% with the high score of 41%.



Fig. 15. Mean frequencies for place preference locations per cat in the *experimental group* (N = 9) of the total observed time of 160 minutes.

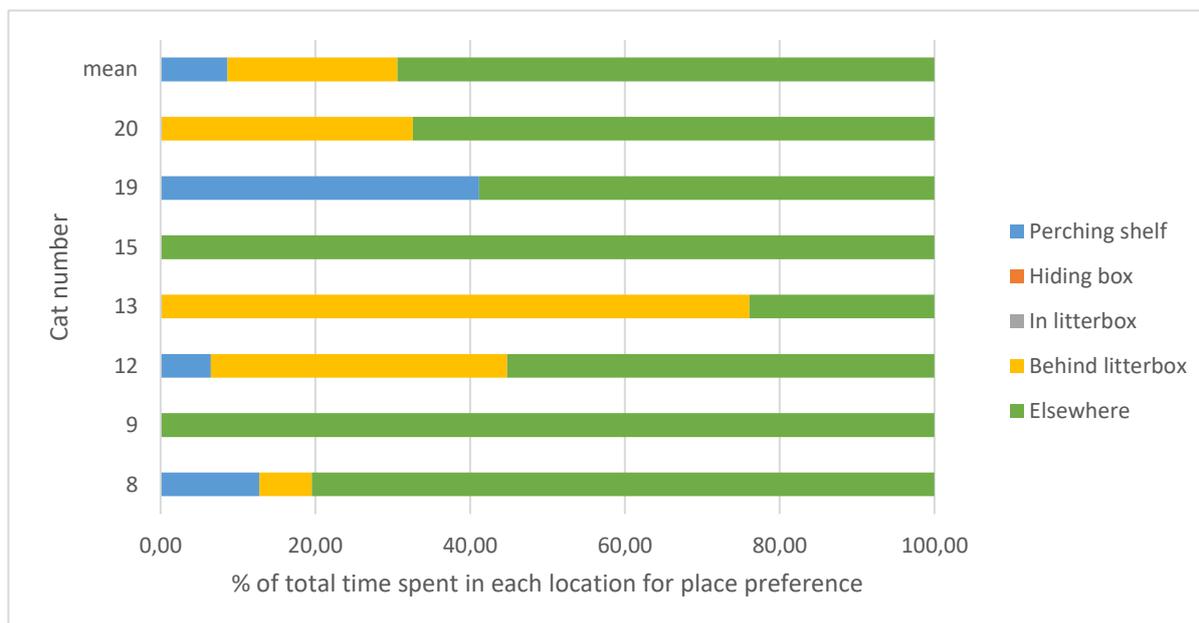


Fig. 16. Mean frequencies for place preference locations per cat in the *control group* (N = 7) of the total observed time of 160 minutes.

Place preference in social housing

Cat 1 was adopted at day 14. Cat 8 suffered from complications due to castration and had to be held in single housing for observation and medication. Cat 9 and cat 12 were kept in single housing conditions for having conflicts with conspecifics when being placed in social housing. Cat 15 had to remain in single housing because of illness and cat 17 was euthanized at day 14. Due to the restricted period of time that was available for data collecting, a follow-up for more than 14 days were not possible for cats 16, 18, 19,

20 and 22. For these reasons, recording place preference in social group housing was restricted to 5 cats (experimental group: cat 4, 7, 11, 14 and control group: cat 13). Mean frequencies for place preference locations on observation days 15,17,19 and 21 are presented in fig. 17. Highest percentages of time were spent outdoors on ground level in peripheral area (G-pa, outdoors = 23%). Second preference for location went to indoors ground level with hiding purpose (G-hp, indoors = 20%), followed by indoors high level with hiding purposes (H-hp, indoors = 17%) like multi-level cat trees. No place preference was seen for high level central (H-ca) and peripheral area indoors (H-pa). Animals spent 39% of total observation time outdoors. Central areas were less visited, both indoors and outdoors as well as high level (H-ca) and ground level (G-ca) compared to peripheral areas. Cats spent 15% of their time on elevated areas like a bench or a cat hammock in the outdoor enclosure (H-pa). Cat 14 spent 100% of the observed time beneath cages, which serves as a hiding place on ground level (G-hp).

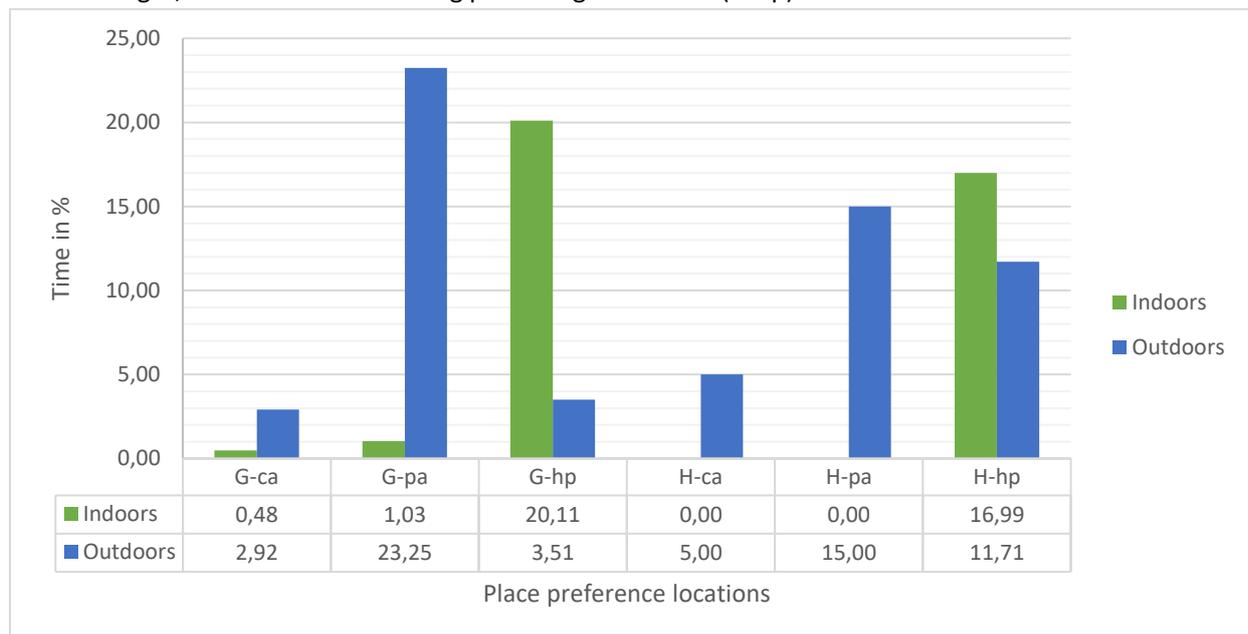


Fig. 17. Mean frequencies of place preference in social housing (N = 5), expressed as a percentage of the total observation period of 120 minutes.

Overall, cats spent a mean of 52% of the observed time in enrichment items with a hiding purpose, either on ground level or high, indoors or outdoors. Non-hiding behavior was registered in 48 % of the observed time. Hiding behavior seemed to increase after observation day 17, at least until day 21 (fig. 18).

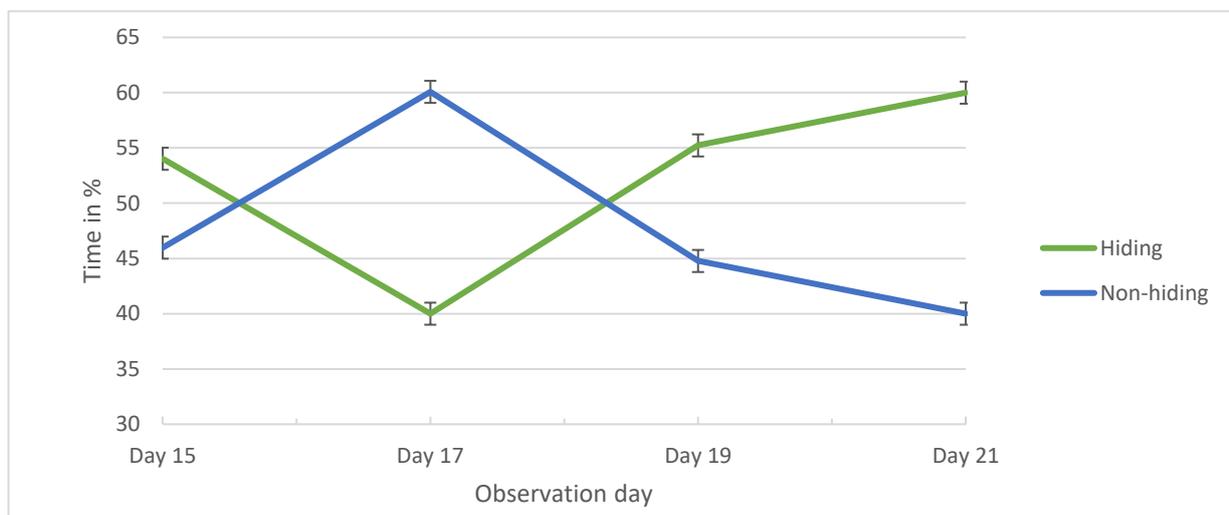


Fig. 18. Relationship between hiding and non-hiding behaviour in social housing (n=5) for observation days 15,17,19 and 21.

Social adaptation score

Social adaptation scores (SAS) were recorded simultaneously with place preference scoring in social housing and were scored for the same 5 animals as mentioned previously: cat 4, 7, 11, 14 (experimental group) and cat 13 (control group) (fig.19). According to these results, only cat 4 and 7 scored a SAS of 1. Cat 11,13 and 14 scored a SAS of 0, since they never went near another conspecific. Cat 4 spent most time in proximity of others (mean = 10% of total observed time of 120 minutes), where cat 11,13 and 14 spent 0% in close proximity.

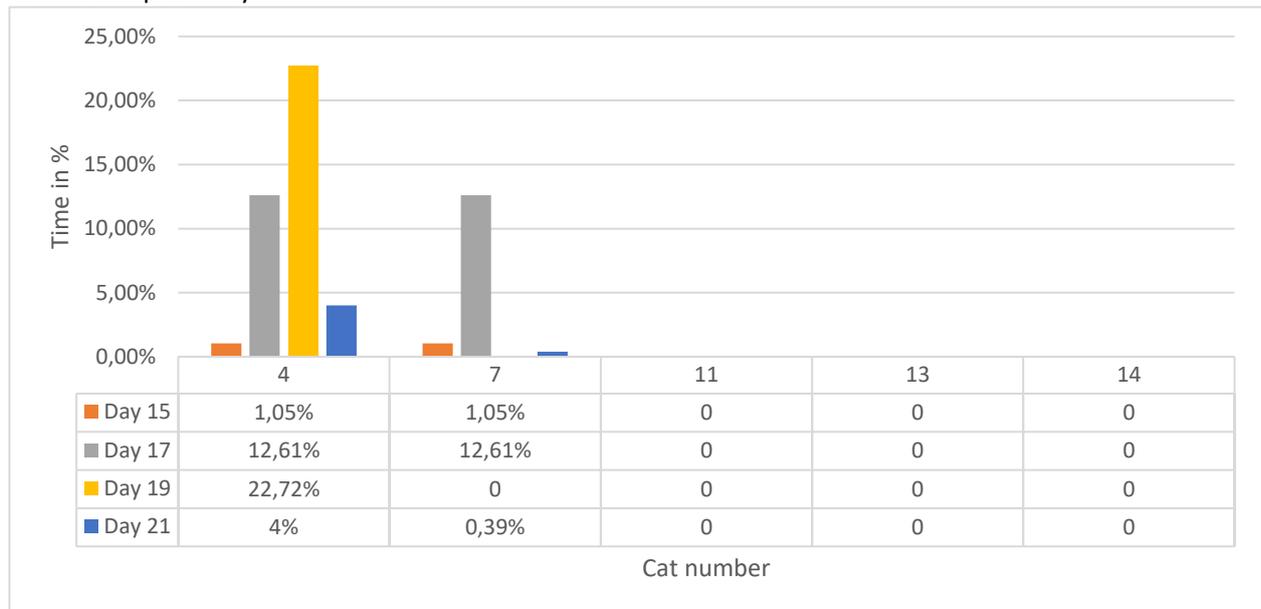


Fig. 19. Mean percentages of time of the total observation time of 120 minutes each focal animal spent in close proximity to a conspecific (N = 5).

Bodyweight

Bodyweights were registered for 8 cats in the experimental group and 7 cats in the control group. Cat 14 (experimental group) was absent on observation day 7 (week 1) and therefore was censored for body weight results for week 1. (Differences in) body weights between week 2 and week 0 were noted for 9 cats in the experimental group and 7 cats in the control group.

The mean bodyweights of cats were 4.32 kg (*SEM* = 0.32) in experimental group and 4.48 kg (*SEM* = 0.16) in control group (fig.20). Shapiro-Wilk tests found normal distributions for the control group ($D(14) = 0.903, p = 0.125, N = 8$) but not for the experimental group ($D(17) = 0.745, p < 0.01, N = 7$).

A linear mixed model was carried out with treatment group, time, gender and surrender type as fixed factors, age as covariate and cat as subject to explore significant correlations between bodyweight, time, gender, age, surrender type and treatment group. After adjustment for multiple comparisons with Bonferroni, no significant differences were located for mean bodyweights between experimental- and control group. A significant positive correlation was found for age with the dependent variable set at bodyweight in kg ($p = 0.008$). Pairwise comparison showed a significant difference between observation day 1 and day 14 ($p = 0.011$) and between day 1 and day 21 ($p = 0.009$) No significant correlations were found for bodyweights between day 1 and day 7 ($p = 0.97$).

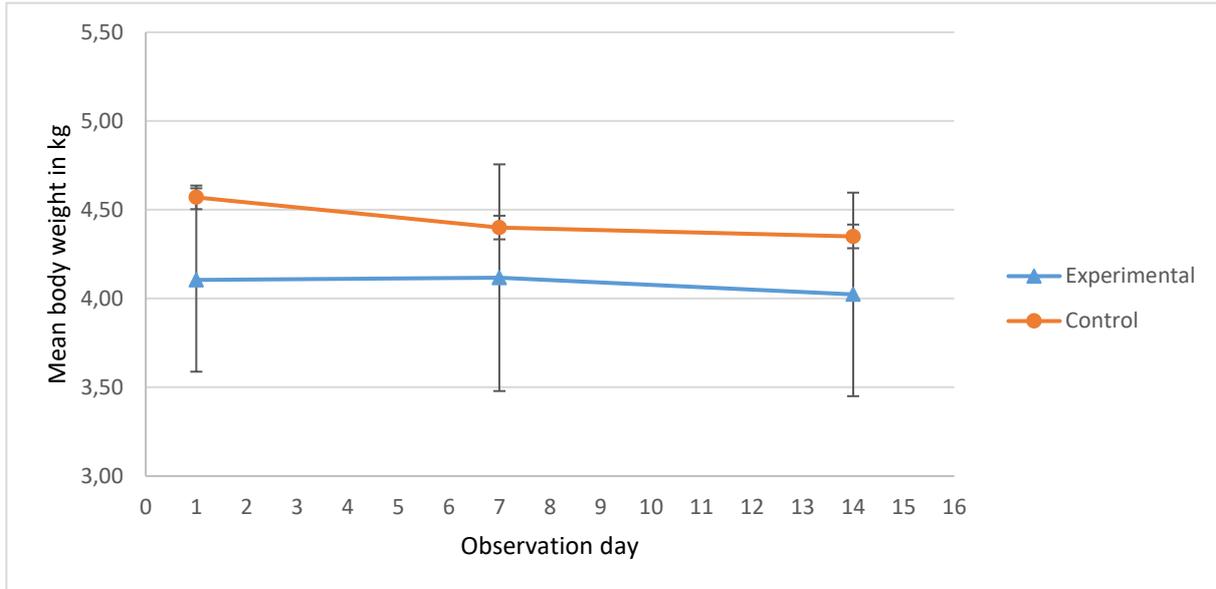


Fig. 20. Mean bodyweights and SEM in kg for both experimental (day 1,14: N = 9, day 7: N = 8) and control (N = 7) group on observation day 1,7 and 14

Body weight losses were registered for 4 out of 8 (50%) cats in experimental group and 6 out of 7 (86%) of cats in control group for the first week. 6 out of 8 cats in the experimental group and 5 out of 7 cats in the control group continued to lose weight in de second week (fig.21-23). During the 14 days period in quarantine, cats in experimental group lost a mean of 2.9% ($SD = 6.38$) of their bodyweight compared to week 0 and for control groups cats this mean was set on 4.7% ($SD = 4.29$)(fig.19). An outlier was formed by cat 18 (experimental group), which had a 17.8% decrease in bodyweight compared to day 1 (fig.20). For both week 1 and week 2, weight losses were reported for 59% of all cats. Overall, 82% of all cats (N = 15) have lost weight during the first 14 days in shelter.

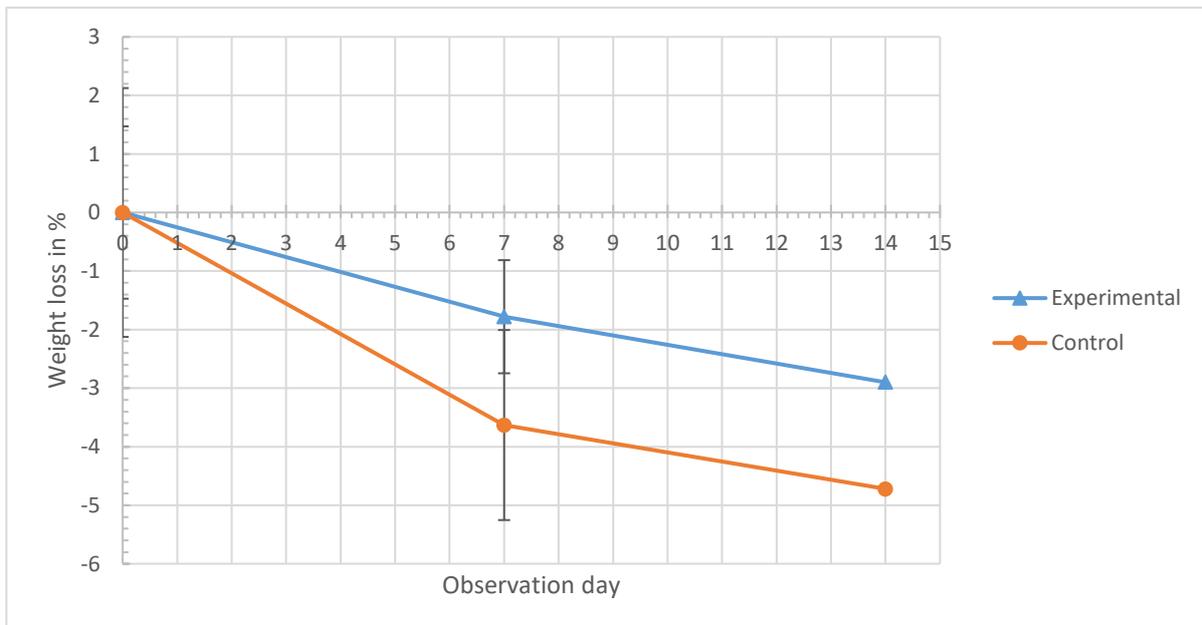


Fig. 21. Mean bodyweight losses at day 7 and day 14 compared to day 1, for both experimental group (day 7: N = 8, day 14: N = 9) and control group (N = 7).

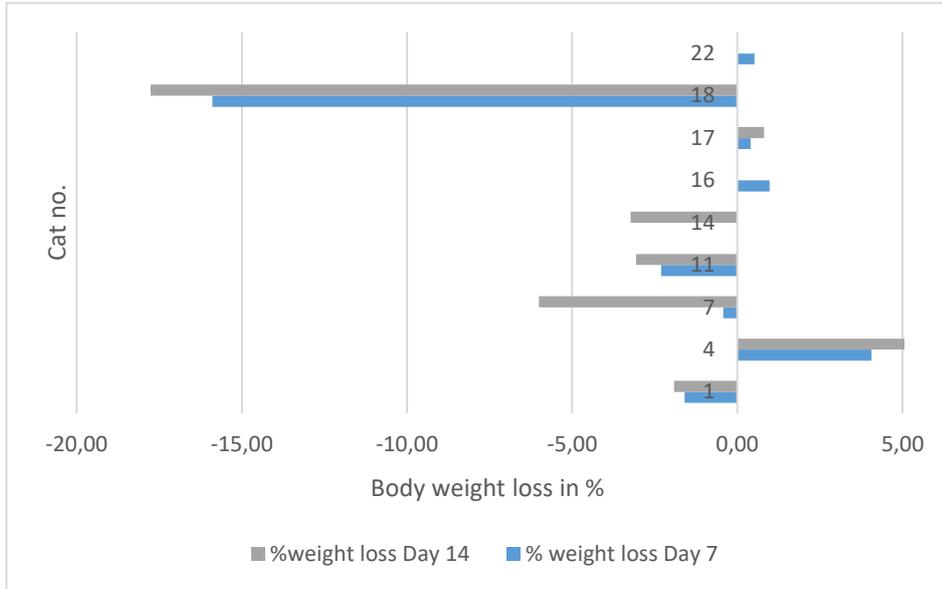


Fig. 22. Individual body weight losses in % at day 7 and day 14 compared to day 1 in the *experimental group* (day 7: N = 8, day 14: N = 9).

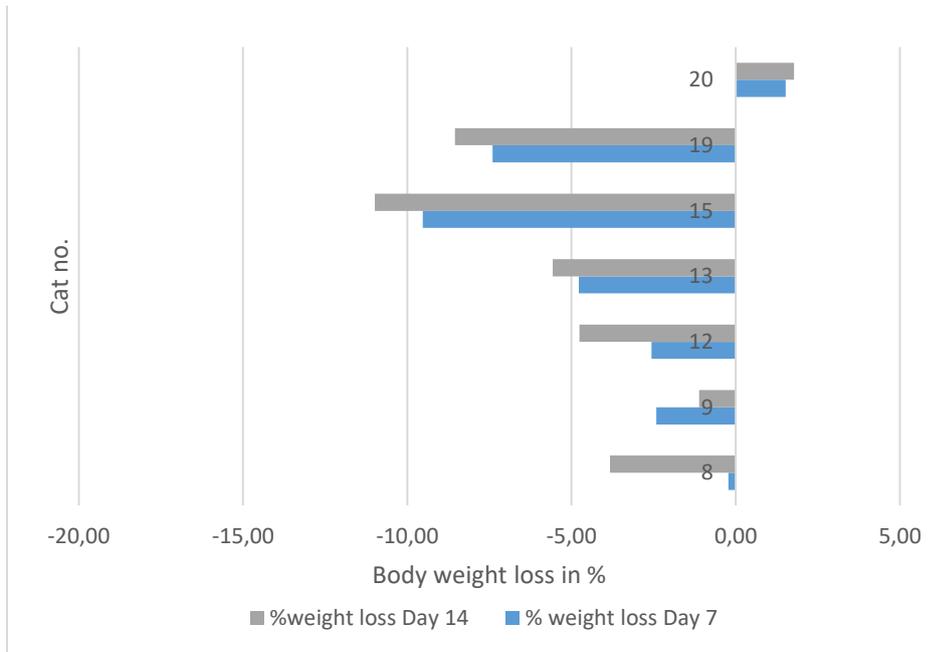


Fig. 23. Individual body weight losses in % at day 7 and day 14 compared to day 1 in the *control group* (N = 7).

Body weight losses were not normally distributed according to Shapiro- Wilk test for experimental group ($D(8) < 0.803$, $p < 0.01$, $N = 8$), but were normally distributed for control group ($D(8) < 0.941$, $p = 0.653$, $N = 7$). To control the assumption of equal distribution before performing a Mann-Whitney tests, homogeneity of variance was tested with the non-parametric Levene’s test with a p value of 0.05. Assumption of equal distribution was satisfied ($F(1,13) = 0.023$, $p > 0.05$), so a Mann-Whitney U test was performed to search for a significant difference between groups. No significant differences were found for week 1 ($U > 16.00$, $p = 0.165$, $N_{1,2} = 15$) and week 2 ($U = 23.00$, $p = 0.563$, $N_{1,2} = 15$) bodyweight losses.

As in Tanaka et al., weight losses during first 14 days in shelter could be calculated as a percentage of initial bodyweight at intake (day 1), which led to the following ranking: (63)(fig.24).

1. No weight loss

2. 0.1 – 4.9% body weight loss
3. 5-10% body weight loss
4. >10% body weight loss

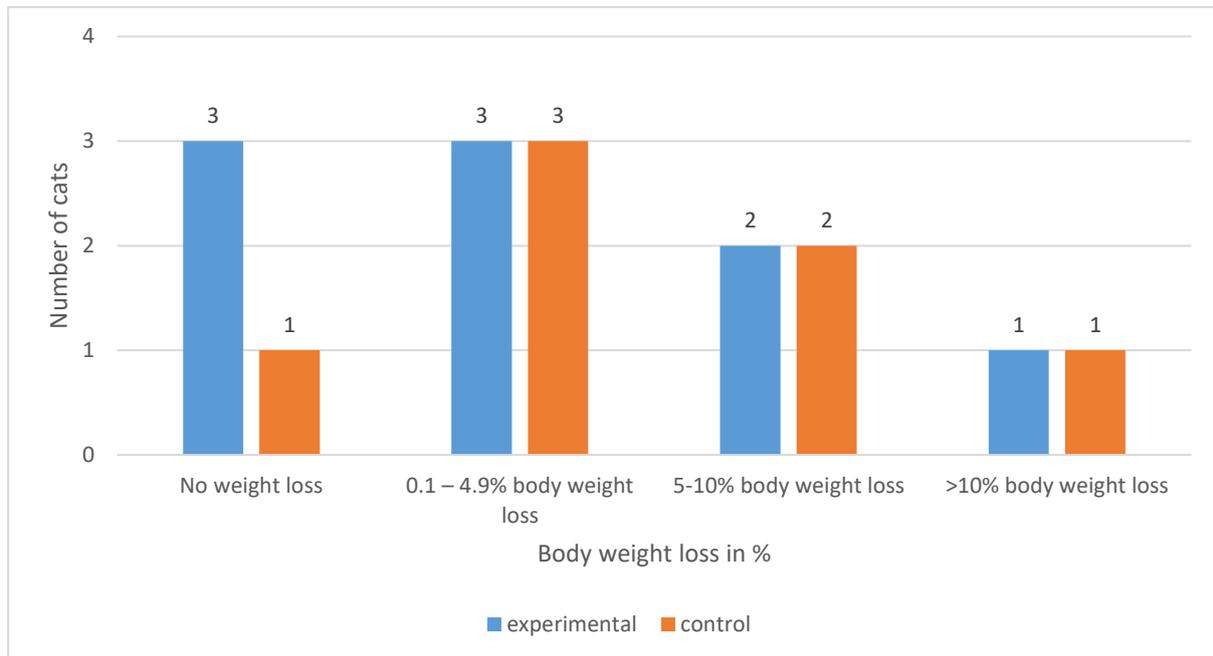


Fig. 24. Classification of bodyweight losses at day 14 compared to day 1 for experimental (N = 9) and control (N = 7) group.

Third week bodyweights were reported for 4 cats in experimental group (M = -0.50, SD = 2.62) and 4 cats in control group (M = -1.20, SD = 1.83) compared to week 2. In experimental group, 2 cats (N = 4) continued to lose weight (cat 1: -2.5%, cat 14: -2.8%). Cat 4 was put on a restricted diet with the purpose of losing bodyweight. For control group (N = 4), bodyweight losses were reported for 3 cats (cat 9: -0.4%, cat 12: -3.7%, cat 13: -1.4%) compared to week 2.

Food- and water intake

The mean food intake for the first 13 days in shelter was determined by calculating the daily food intake in grams per cat and was reported as a percentage of the daily amount of food that was offered to that particular animal (fig.25, 26). Shapiro Wilk test found no normal distributions for both treatment groups (experimental group: D(114) = 0.608, $p < 0.01$) (control group: D(91) = 0.644, $p < 0.01$). No significant differences were found for median food intake between the two treatment groups after performing a Mann-Whitney U test (U = 28.500, $p = 0.749$). Spearman's Rho correlation test was used to test a possible inter-animal relationship among body weight and food intake. Within groups, no significant correlations were found.

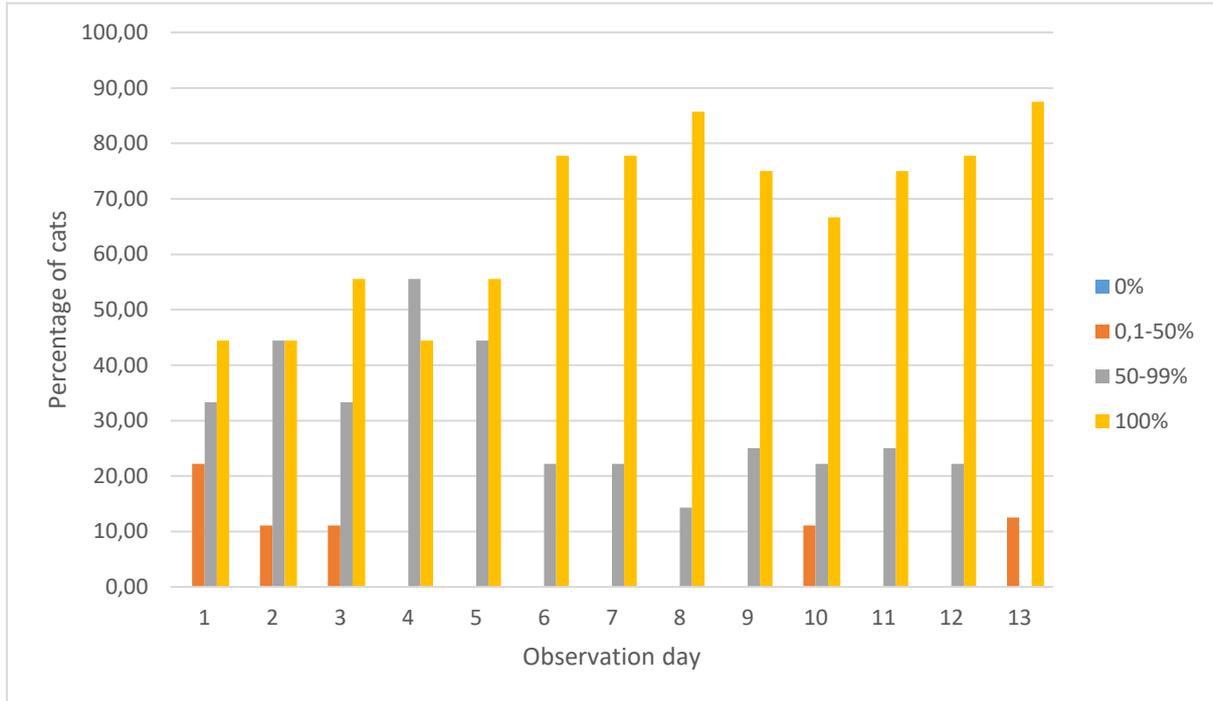


Fig. 25. Ranked mean daily food intake for mean percentage of cats in the *experimental group* on each observation day (N = 9).

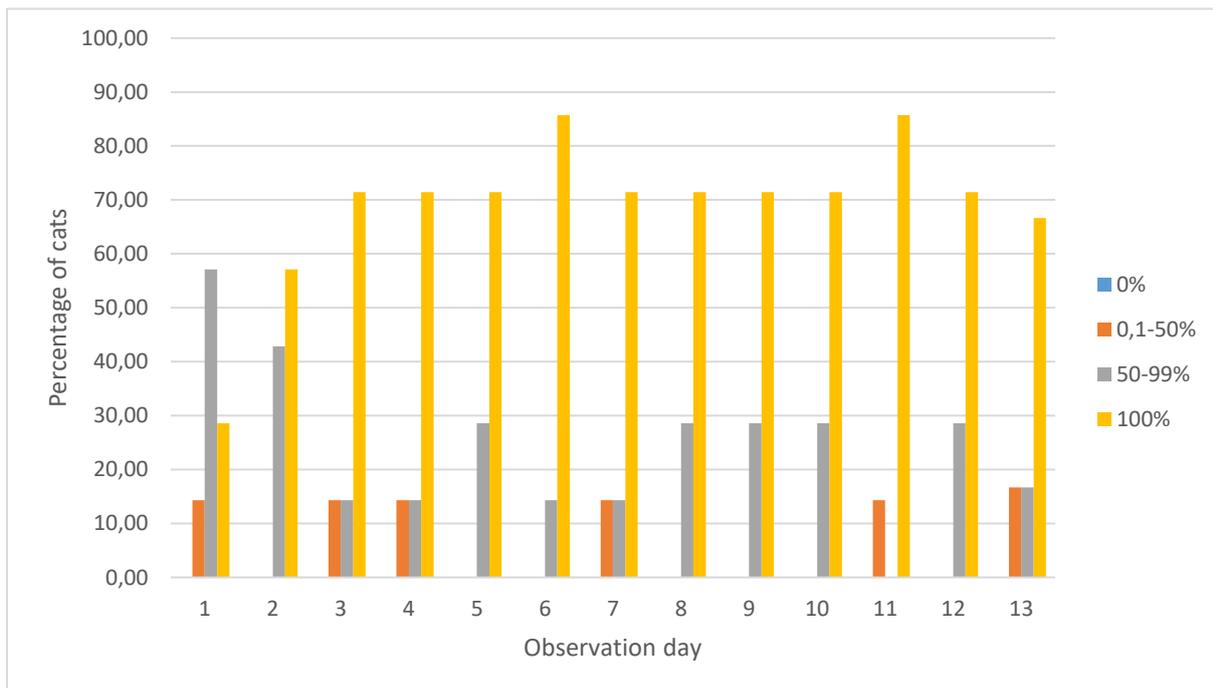


Fig. 26. Ranked mean daily food intake for mean percentage of cats in the *control group* on each observation day (n=7).

For both groups, mean food intake was lowest on day 1 and increased on day 2. Cats in the experimental group showed a median food intake of 89,8% (IQR = 11.4), cats in the control group 89.2% (IQR = 6.9)(fig. 27).

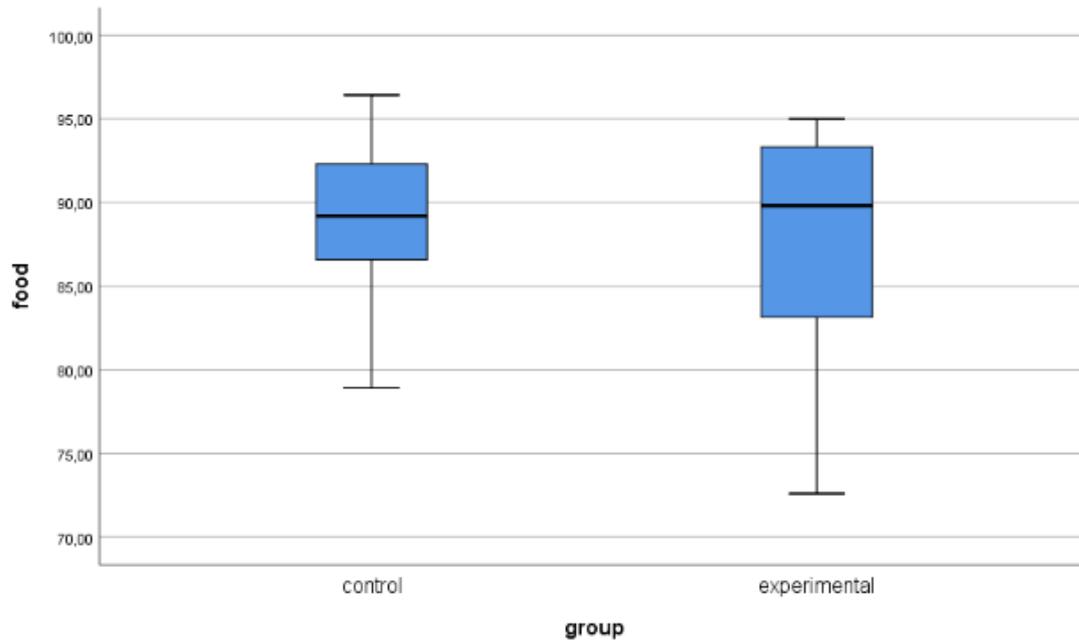


Fig. 27. Median (\pm IQR) food intake in % of total amount that was offered per cat in the first 13 days in shelter for both control group (N = 7) and experimental group (N = 9).

Average daily water intake in experimental group cats was 100.8 ml with a median of 106.1 ml ($SEM = 5.1$, $IQR = 24,37$) and in control group cats 105.9 ml with a median of 112,5 ($SEM = 6.8$, $IQR = 27,18$) (fig.28). Normal distributed data were found for the control group according to a Shapiro-Wilk test, but not for the experimental group (experimental group: $D(14) = 0.848$, $p = 0.021$), control group: $D(14) = 0.878$, $p = 0.055$). Mean water intake was at its lowest at day 1 in both research groups (experimental group: 50,0 ml, control group: 37,86). After performing a Mann-Whitney U test, no significant differences were located between the experimental group and the control group.

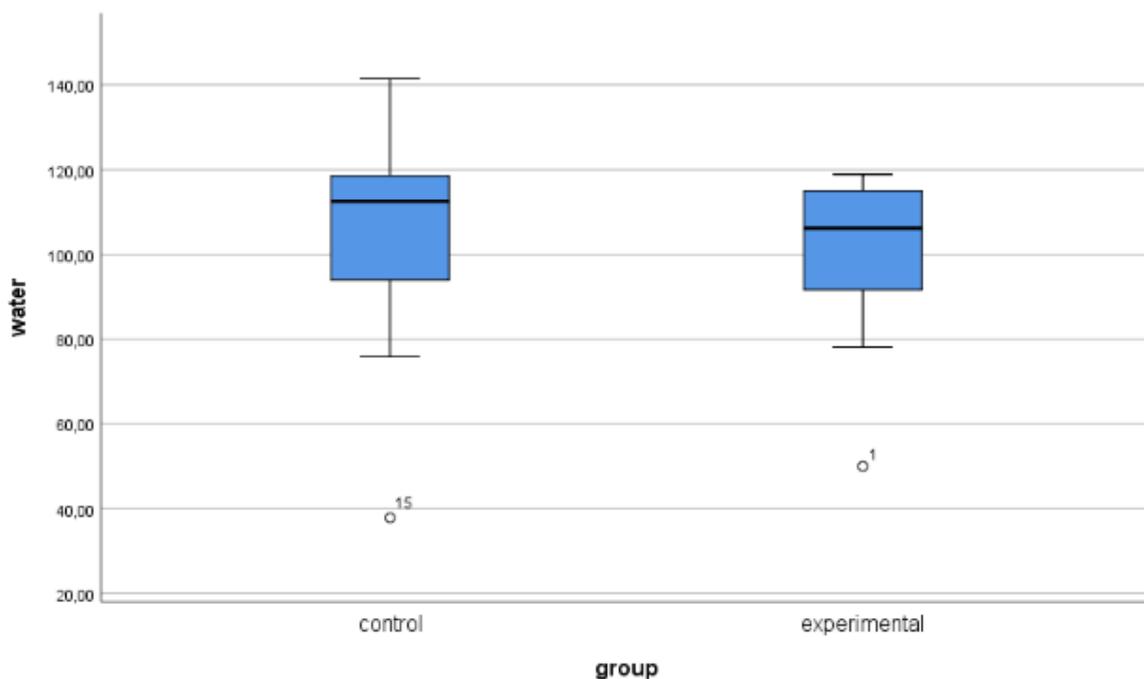


Fig. 28. Median (\pm IQR) daily water intake (ml) for experimental group (N = 9) and control group (N = 7).

As to elimination 5 out of 9 (56%) cats in experimental group did not urinate the first 24 hours in shelter, 2 out of 7 (29%) cats in control group did not urinate first 24 hours. Elimination of feces in the first 24 hours; 5 of 7 (71%) cats in control and 6 of 9 (67%) cats in experimental group did not eliminate first 24 hours.

fURI

Cat 17 had the highest fURI score at intake (score of 6), but because she was diagnosed with a tumor in the nasal cavity at observation day 14, she was excluded for overall fURI data analyzing.

Overall, the average fURI score of the experimental group for observation day 1,2,3,5,7,9,12 and 14 was 1.63 (*SD* = 1.39) with a median of 1.07 (*SEM* = 0,49) and for control group 2.06 (*SD* = 1.59) with a median of 1.29 (*SEM* = 0,60) (fig. 29). A Shapiro-Wilk test showed normal distributions for fURI scores at day 1 and day 9, but not for day 2,3,5,7,12 and 14. 100% of the included cats had or developed signs of feline upper respiratory tract infections during the first 14 days in shelter. At day 1, 6 out of 8 cats in experimental (*M* = 1.63, *SD* = 1.41) and 6 out of 7 cats in control group (*M* = 2.14, *SD* = 1.95) already showed signs of fURI. At day 7, all cats had developed signs of fURI with a mean score of 1.88 (*SD* = 1.36) in experimental group and 2.14 (*SD* = 1.86) in control group (fig.30).

Spearman’s Rho correlation test did not show any significance between treatment group, surrender type, gender and fURI scores, but did show a significant positive correlation (2-tailed) for higher fURI scores with higher age (*r_s* = 0.582, *p* = 0.023). Mann-Whitney U test showed no significant differences for mean fURI per cat and overall mean fURI score (*U* = 20.000, *z* = -0.927, *p* = 0.354) between both groups. A Friedman’s ANOVA was performed to find out if fURI scores differed significantly per cat between the observation days for both experimental as control group. No significant differences were detected for experimental group (χ^2 (7) = 5.944, *p* = 0.546, *N* = 8) and control group (χ^2 (7) = 8.796, *p* = 0.265, *N* = 7).

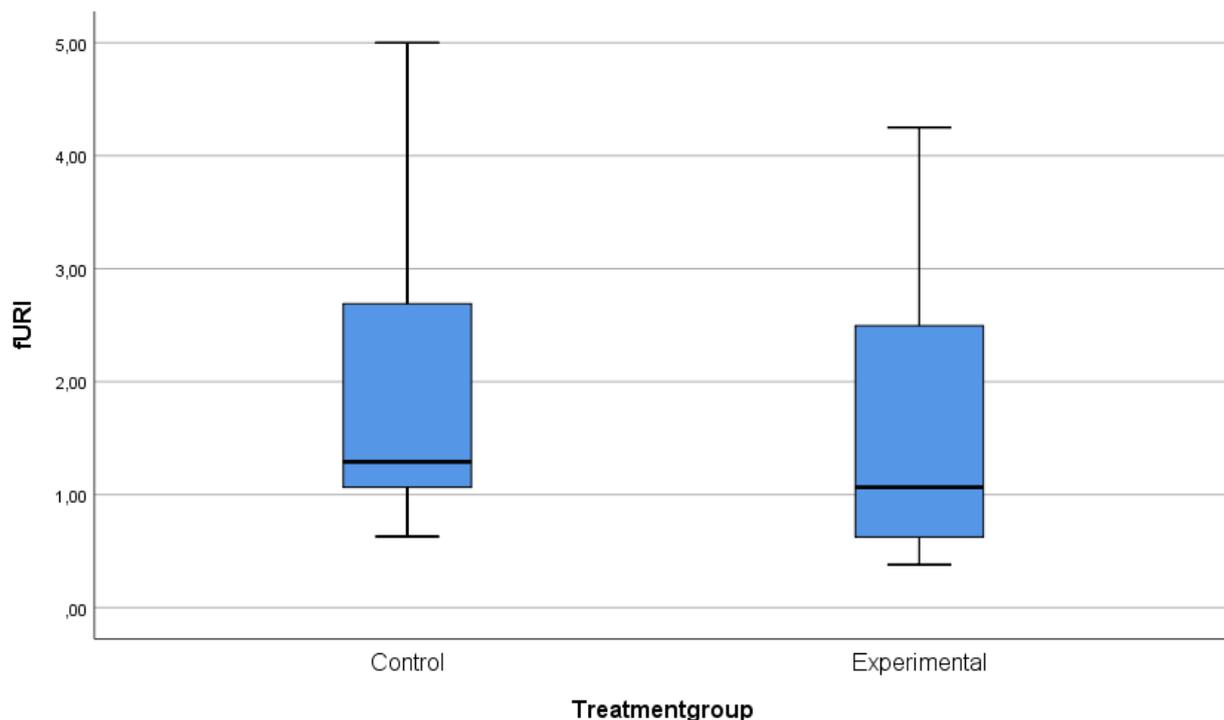


Fig. 29. Median (± IQR) fURI scores during observation day 1,2,3,5,7,9,12 and 14 for both control group (*N* = 7) and experimental group (*N* = 8).

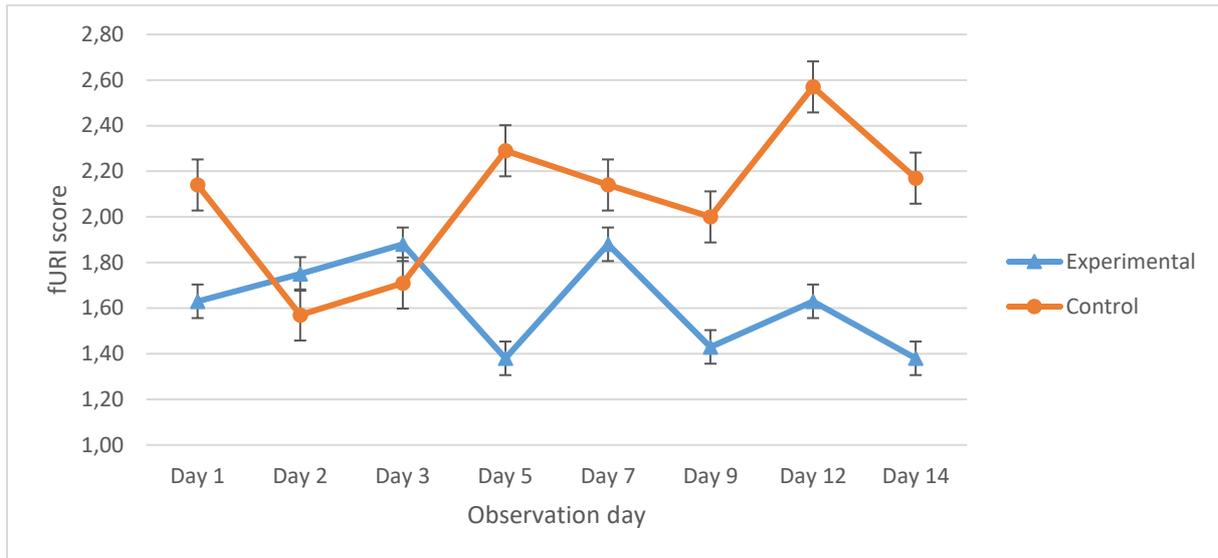


Fig. 30. Mean daily fURI scores of the experimental group (n=8) and control group (n=7) for observation days 1,2,3,5,7,9 and 14.

Adoption rates

15 out of 16 (94%) cats were adopted. One cat (cat 17) from the experimental group had to be euthanized at day 14 since she was diagnosed with a tumor in her ethmoid sinus. Mean adoption rates for the experimental group were 29.1 ($SD = 31.9$) days and for control group 27.7 ($SD = 30.5$). A Shapiro-Wilk test for the adoption rates of both experimental and control group was conducted and both groups were not normally distributed (experimental group: $D(9) = 0.824, p = 0.039$); control group: $D(7) = 0.674, p < 0.01$). Mann-Whitney U test did not find any significant difference between mean adoption rates between treatment groups ($U = 30.00, z = -0.159, p = 0.874, N_{1,2} = 9,7$). Subsequently, a Spearman's Rho correlation test was carried out to investigate a possible relationship between adoption rates, gender, age, surrender type and treatment group. No significant correlations were found.

A Cox Proportional Hazard Regression analysis was conducted to detect potential variances if chance of adoption is higher for cats deriving from experimental group or control group. Cat 17 obtained a censored status and the confidence interval was set on 95%. No significant correlations were found ($RR = 1.058, 95\% CI, p > 0.05$).

Fig. 31 shows a median adoption rate of 21 days for experimental and 22 days for control group and was studied for outliers. Outliers were formed by cat 15 (control group), who had the longest stay in the shelter (95 days). This cat was diagnosed with infections of FIV and the dermatophyte *Microsporum canis* after the 14 days in quarantine and had to be held in shelter for medical treatment of the skin condition, staying unavailable for adoption for several weeks.

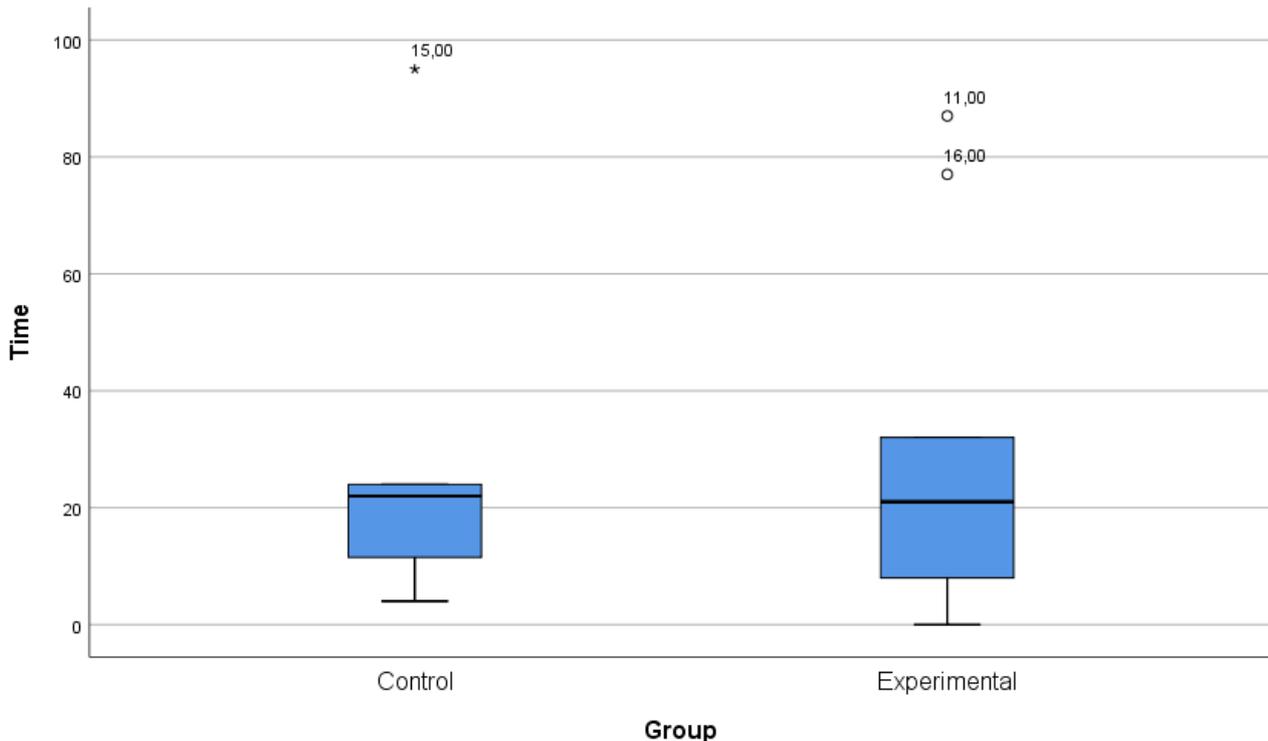


Fig. 31. Median (\pm IQR) adoption rates (in months) of control (N = 7) and experimental group (N = 8). Outliers are formed by cat 15 (control) and 11 and 16 (experimental)

DISCUSSION

The aim of this study was to investigate the importance of environmental hiding enrichment for reducing stress and preventing an impairment of the animal's well-being in newly arrived shelter cats. In order to achieve this goal, locations for place preference in confined areas were determined and body weights and the prevalence of clinical signs of fURI were registered in newly arrived cats during the first 14 days in individual housing in the quarantine area and the first 7 days in consecutive social housing in a Dutch animal shelter. Based on previous research, we expected that cats with a hiding box showed the least decrease in body weights and represented the lowest fURI scores and recovered more quickly during their stay in the quarantine area. For social housing in units we expected cats who spent their time in close proximity to other cats showed a (faster) decrease in stress levels reflected by the least decrease/most increase in body weight, the lowest prevalence of clinical signs of fURI and/or spent the least amount of time in concealed types of hiding enrichment and/or on elevated levels, measured by scoring place preference in social housing units. Based on results from previous studies we expected that measuring bodyweight losses in shelter cats will act as a reliable and applicable method for shelter staff members to recognize stress. Finally, we expected that cats originating from the experimental group (the "hiding box" group) in quarantine housing spent less days in the shelter than cats deriving from the control group, reflected by higher adoption rates. Most important results include:

1. Cats without access to a hiding box spent significant more time elsewhere than cats who did have access to a hiding box. More than half of the total observed time was spent in the box.
2. In social housing units, cats spent most of their time outdoors on ground level in peripheral areas followed by a preference for being in an enrichment item with hiding purpose. Central areas were least favorite.
3. Significant differences in bodyweights were registered between day 1 and day 7, and between day 1 and day 21.
4. 13 out of 16 cats lost weight during the first 14 days in shelter and 5 out of 8 cats continued losing weight after leaving the quarantine area. Although, there were no indications for a significant difference between the treatment groups regarding to bodyweight losses, mean bodyweight loss in the control group (-4,7%) was almost twice as high as in the experimental group (-2,9 %).
5. 100% of the cats had or developed signs of fURI during the first 14 days in the shelter and a higher fURI scores were significantly and positively correlated with older cats (higher ages in months).
6. A positive SAS was scored for 2 out of 5 cats.
7. No significant differences were registered between the experimental group and the control group regarding to bodyweight loss, food intake, water intake, fURI score and adoption rates

Place preference in individual housing

When cats are stressed – for example when they are confronted with new stressors for being placed in a new environment - they tend to perform more hiding behavior. This could explain why cats from the experimental group in the present study spent 54% of the studied time in their hiding box (6,51). These results are consistent with the previous studies of Vinke et al. and Selman et al where cats used their box respectively 55% and 59% of the total observation time (54,68). Cat stress score (CSS) used in previous studies to assess stress in shelter cats, showed a higher mean CSS in control group cats compared to experimental group cats and CSS decreased until a stable CSS was reached at day 5, whereas mean CSS in experimental group needed 3 days to reach a stable level (68). The 33% decline in time spent in hiding box on day 2 compared to day 1 agrees to the previous study of Selman et al. where a fast decrease in stress scores was found on day 2 in the experimental group and to McCune (1992) who found highest scores of stress when placed in a new, confined area in the first 24 hours (54,35). Although a decrease was also seen in control group cats, it was more gradually and slower compared to the pronounced decline in experimental group cats. Speculatively, mean stress scores when measured with CSS would be highest at day 1 in experimental group and decrease more rapidly than CSS in control group.

Several studies found that cats that did not have access to a hiding box spent a significant higher amount of time behind the litter box as was also the case in the current study (6,33,52,68). Time spent behind the litter box in control group cats (24%) was a lot higher every day than observed in the experimental group (3%) and percentages were consistent with findings in Selman et al. (23% for control group cats and 2.4% for experimental group cats) (54) Crouching behind the “only cage furnishing that offered some concealment” is described as an attempt at hiding and could explain why cats who did have access to a hiding box, did not have the need to search for alternatives for hiding (6). However, this type of hiding behavior did not provide a significant decline in stress levels (6). Another type of alternatively hiding behavior was observed in cat 13 (control group) which searched for concealment under a towel, suggesting a higher motivation of performing hiding behavior to avoid stressors, than being on a comfortable surface provided by material of the towels (48).

The fact that control group cats spent more time on the shelf than experiment group cats strongly suggests the need of a cat to use elevated areas and implicates the importance for cats to use vantage points to monitor their surroundings (48,56). Cats with access to a hiding box spent more time in the box than on the shelves suggesting the perching shelf serves as an “environmental enrichment item that alleviates a negative state rather than promoting a positive one” and hiding boxes providing the “most benefit”. However inter-animal varying is high, suggesting individual variation between cats, these results have been supported by Ellis et al. 2017 (15,16). This variability between cats also reflects in preference for “being elsewhere”. In control group cats, this is the most preferred location and significant different than control group. In experimental group, “being elsewhere” is most preferred after “hiding box”. These finding are consistent with Vinke et al., but in contrast with Selman et al. were mean time spent on the shelf was higher than being elsewhere (54,68). Dissimilarities in results could be due the once daily 20 minutes observation for place preference may be too short to produce a representative sample of the day.

Multiple, separate observations per day could improve reliability of the results because the chance of overestimating certain place preferences, like sleeping at one place for the full 20 minutes of observed time, could be reduced, like was performed in Suchak et al. (62). However, comparable results are found in previous study about mean frequencies for place preference, suggesting used time window and duration of observing was done right (54,68).

Place preference in social housing

Although group housing with conspecifics could offer benefits for cats, for example companionship, socialization, play and more space per cat, it also increases risks of exposure to infectious diseases, could elevate stress and anxiety and makes monitoring of individuals more challenging (41). The need to hide and perch are important and negative contact between cats were observed more frequently in situations where cats are unable to retreat to perching areas or hiding spaces. In addition, the restricted area could make it hard to hide from others or avoid conflicts leading to negative encounters (20,42). Gourkow et al. however did not report higher CSS for cats in group housing which could be explained by offering many types of enrichment to ensure cats can avoid other cats (20). In other research, group housing lead to higher stress levels than individual housing, especially when cats were poorly socialized with conspecifics (31,35,42). In this present study, a mean of 52% was spent in enrichment items with a hiding purpose, underscoring the importance of hiding behavior. These finding were supported by Ellis et al. 2017, who reported that cats allocated most in their time to compartments that contained hiding enrichment when placed in a novel surrounding (16). Ellis et al. reported a preference for concealed cat igloos in cats with low stress scores and use of upper platform of a multi-level cat tree, analogue to H-ca in present study, with high stress levels (16).

After hiding enrichment, being in peripheral areas on ground level was preferred. Previous results report that stray cats were found to avoid “large” empty space because they were more suspicious for danger, although Rehnberg et al. discovered similar results in owner-surrendered cats (16,47). Avoidance of open spaces by cats it supported by current results, for both ground- level as high level central areas were least favorite.

Because of the small sample size (N = 5), mean place preference could be influences by outliers and results should be interpreted with caution. For next studies, repeating this setup with a larger sample size would be interesting for locating any benefits of a hiding box in the first 14 days in quarantine on place preference locations in social housing units. Since in this study an increase in hiding behavior was

observed at least from day 17 until day 21, it would be interesting to observe for longer periods to detect developments in hiding behavior after a longer stay in the shelter.

Social adaptation score (SAS)

Time spent in proximity of conspecifics could be used as a measure for tolerance towards other cats in group housing (62). In this current study, 2 out of 5 cats studied for SAS had a SAS of 1, meaning they spent time in close proximity to other cats. A large variation per cat was seen for SAS. Keeping distance from other cats suggest the wish to avoid close cat-cat contact, but also could be a result of inactivity and therefore suppression of behavior moving away from potential stressor(20,62). Alternatively, the obesity of cat 4 could lead to a decreased activity. Proximity in this particular case could be mistaken for tolerance rather than preference of being near to conspecifics (62). Cat 7 got approached by cat 4, influencing its SAS and making it difficult to differentiate between tolerance or a positive event due to social contact. Another explanation could be that cat 4 and 7 stayed in the same quarantine room for 13 subsequent days. Although they were housed in different cages, maybe smell and sound could have caused a gradually adaption to each other.

Differences in sociability towards conspecifics could also be assigned to surrender type, in which owner surrendered cats seemed more sociable towards other cats than strays (62), in this study sample sizes were too small to discriminate between strays and relinquished cat and a possible correlation to their SAS.

Data for longer periods with larger sample sizes and differentiate between gender, surrender types and positive versus negative approaches is needed to get more insight in social structure and stress behaviors in group housing situation.

fURI

All cats with any signs of upper respiratory tract infections were registered as being infected, according to scoring method of Dinnage et al. which resulted in a 100% of all cat developing signs of fURI during the first week of arrive in shelter (11). Although variation in scoring was kept as low as possible, some bias by investigator was inevitable for this scoring method was quite subjective. Serous nasal and ocular discharge was registered as being infected, but since these signs are not very specific, fURI may be overrated. In this study, this scoring method was interpreted in a different way than in Dinnage et al. in order to produce a total cumulative severity score for fURI per cat, rather than allocated them to a specific group (score 0,1,2 or 3) to avoid generalization for all signs(11). Even though this method might not be fit for establishing presence of a current fURI, variations between treatment groups in time and severity of fURI scores reflected in the degree of fURI score may be become visible and will give insight in development of disease.

In this present study, higher fURI scores were positively correlated with older cats, which is in accordance with findings that a higher age serves as a risk factor for development of upper respiratory tract infections, although very young and very old animals are most vulnerable and were excluded from this study (11,14,21). Cats in the experimental group showed a mean fURI score of 1,62 which was lower than the mean fURI score of the control group (2.14), although differences were not significant. However, mean ages in experimental group were higher than mean ages in control group. This differences in mean fURI scoring could be explained by reducing stress in experimental group by offering a hiding place.

Correlations were not found between surrender type and fURI score. In other studies higher fURI scores are associated with relinquished cats which agrees with results of Dybdal et al. that indicate that cats surrendered by their owners became affected sooner by upper respiratory tract infections than strays

(12). Although stray animals were at greater risk of exposure due to lack of vaccination and (in most cases) incomplete nutrition, relinquished cats were at a greater risk of getting ill sooner (12). The lower CSS that was found in Dybdall et al. in stray cats, suggesting strays adapted sooner to changes in environment and were better prepared to fight infection. This is in contrast with Dinnage et al. which found stray adult cats were at significantly higher risk of fURI compared to relinquished cats. (11).

Quarantining newly arrived cats is based on incubation times of diseases which purpose can be that clinical signs will develop while still in quarantine and preventing adding sick animals to the healthy population of cats in group housing (11). Time to develop fURI seems to reflect the incubation period of contributing viruses and are 2-6 days for FHV and 2-10 days for FCV. Time for reactivation of latent infection is seen in stressed cats at 4-11 days for FHV (7,18_b,27). These facts could explain the 100% affected population in first 14 days in new environments. Data from this study suggest a risk for developing signs of fURI when entering the shelter. Whether these results are due to the stress of new environment, or due to reactivation of previous infection, or because factors in shelter situation are making the individual more susceptible for (new) infections is not yet fully understood (2,53). Although, previous research described a latent phase of FHV with periodic episodes of virus shedding which could be induced by stress caused by a new, unfamiliar environment and suggesting reactivation of FHV in a population by a newly introduced, latent infected cat is an important risk factor in the epidemiology of fURI (18_a). Various studies reported findings of hazard of developing fURI within 50 days of arrival of a new cat, but hazards decreased with time and peaked until 13-20 days and then declined (2,14). For next studies it would be interesting to investigate fURI scores during longer stay in shelter (longer than 14 days) and what happens when a change of housing takes place e.g. from individual to social housing.

Body weight

Body weight losses were reported for 81.3% of all cats during first two weeks in shelter. Mean body weight loss in experimental group was -1.8 kg and for control group cats a mean weight loss of -3.6 kg happened. Tanaka et al. reported 59% of cats (N = 58) lost weight during the first week and weight loss in 62% of the cats (N = 39) in the second week in shelter (63). Remarkably, this present study also revealed a weight loss of 59% of the cats during week 1 (experimental group: 4 out of 8 cats, control group: 6 out of 7 cats). This same percentage was found in the second week in shelter.

On day 21, body weights were registered for 8 animals (experimental group; N = 4, control group; N = 4). 5 subjects continued to lose weight. For experimental group cats: Cat 4 was put on a diet for being severely obese and his weight loss was intentional. Cat 14 was continuously hiding beneath cages and the loss of body weight could be attributed to a decreased food intake related to stress. The other two cats originating from the experimental group (both male strays) gained weight (respectively 0.7% and 2.6%) compared to day 14. Cat 9, 12 and 13 (control group) continued to lose weight compared to day 14 (respectively 0.4%, 3.7% and 1.4%). Cat 15 stayed in individual housing and gained 0.6% of its weight compared to day 14. Despite the small sample size of follow up after day 14, it is remarkable that 5 out of 8 cats continued to lose body weight after being placed in subsequent social housing conditions, except for cat 15 who stayed in the same quarantine cage. This indicates the change of environment involves new stressors through unfamiliar routines, conspecifics and handlers (6). Weight loss could also be the result of a more stimulating environment with more room to walk, climb and more toys available which could increase activity (15,64). Pointedly, most cats who continued to lose weight derived from control group. For next studies it would be interesting to repeat this setup with a larger sample size in order to identify relationships between treatment group and weight loss for a longer period of time, to determine when body weights stop decreasing in a shelter setting.

Food- and water intake

During the first 24 hours stay of a newly arrived cat in confined area, inhibition of eating, drinking, urinating and defecating is seen in many cats and is an indicator of stress (59). Similar events have been noticed in Stella et al. 2014 in caged cats, but also in owned cats and laboratory cats (25,47,57,59). In Tanaka et al. 34% of all cats did not eat on day 1 and 84% of the cats that did not eat on day 1, did not eat on day 2 either (63). This is in contrast with the present study, were 100% of all cats did eat on day 2, although lowest intake of first 14 consecutively days in shelter was at its lowest point at day 1 and 2 for control group cats. In Tanaka et al. cats did not had hiding options or elevated areas in the cage, which could explain lower food intake due to higher stress score for food intake and stress scores were negatively correlated (63). This is consistent with findings in other studies, were deprived food intake is caused by lower appetite due to higher stress (6). Food intake at day 1 is for both control as experimental group at its lowest point and raises at day 2 with a faster recovery for experimental group than control. These results are similar with Stella et al. 2014 were appetites recovered faster in cats housed in a managed room versus an unmanaged room (58). Cats in unmanaged rooms were confronted with unpredictable daily disturbances and inconsistent routine which underscores the importance of consistent management in shelter situations.

Food intake was to be found higher at morning rounds, suggesting cats did eat more at twilight or night hours, consistent with other studies (29,60). However, cats in shelter situation are subject to staffs routine and therefore may be more active during day hours in reaction to human activity (16). Ellis et al. 2013 found that greatest amounts of food intake were initiated around feeding times and that the percentage of time spent at eating increased with time (57).

Cat 14, 17, 18 and 22 were spending > 50% of the overall observed time of 160 minutes during the first 14 days in quarantine, in the hiding box and these cat had the lowest overall food intake percentages. A possible explanation could be inhibition of food intake because hiding behavior has a higher motivation than eating, for food was not directly offered inside of the hiding box.

50% of all cats in this present study did not urinate and 69% did not produce any feces during the first 24 hours in shelter. No uptake of food could explain this, while also the fact that many cats will avoid use of litterbox when these are located in close proximity like in confined caged areas or when substrate is unknown and/or aversive may play a role (71).

Adoption rates

As in Selman et al. and Kry and Casey no significant differences were detected for adoption rates in the experimental as compared to the control group, although mean adoption rate of experimental group cats was slightly higher than control group cats (33,54). Results could be influenced by outliers. Cat 1 (experimental) had the shortest overall stay in shelter, for this cat reservations were possible for potential owners when still in quarantine. Longest stay in shelter was registered for cat 15 (95 days), but this cat was diagnosed with FIV and was suspected of an infection with dermatophytosis and therefore unavailable for adoption until circa 50 days after leaving quarantine area. After recovery, cat 15 was posted on social media which may positively influences adoption rate because of reaching a larger subset of potential adopters (70). It also can be influenced by compassion as a motivation to purchase a certain cat (20,33). Cat 17 had to be euthanized after quarantine period for having a tumor in the ethmoidal cavity. The relatively long stay of cat 11 (from the experimental group) could be explained because of his black coat, which some studies showed is the least favorable coat color (32,55,69). However, the other black cats did not showed prolonged adoption rates (cat 1; 0 days, cat 7; 11 days, cat 18; 25 days and cat 9; 22 days) and probably could be explained for its ambivalent personality (33,70). Cat 16, a male

neutered, red-white coated cat with the adoption rate of 77 days could be explained by his age which was 111 months for elderly cats are less likely to be adopted (52).

Because characteristics like coat color, coat length, breed and age are predetermined and not subject to changes, other methods could be used to improve adoption chances like focus at behavioral and personality traits (15). Cats behavior towards potential adopters is mentioned as an important factor as reason for adoption, reflected in social greeting behavior and/or a direct interaction between cat and potential owner and level of activity (17,55,69). Hiding boxes could restrict visibility for audience which is not so important in quarantine housing but in the subsequent individual or social housing, cats could be overseen by potential adopters when hiding in enrichment items. However, hiding boxes could potentially increase adoption chances because cats with hiding opportunity have lower stress scores which positively influences social behavior towards humans, although more research is necessary to support this statement. Fantuzzi et al. mentioned cats on higher tiers are much more viewed than cats in lower tiers, which increases adoption chances (17). Placing of hiding enrichment could therefore be restricted to higher areas in order to promote visibility for potential owners and could be an interesting an easily applicable adjustment in management (17,55).

Cat's welfare implications

In order to reduce stress and improve welfare in domestic cats that are being placed in a new environment, it is important to acknowledge inter-animal variations. Enrichment items and types of housing (social versus individual) should be compatible with the behavioral styles of each individual. It may be interesting to search for an easy applicable method for making assessments/husbandry protocols for handling, housing conditions, interaction with other cats which could be included in management in order to positively influence behavior, immune status and therefore reducing signs and chances of illness and improving adoption chances. While hiding behavior is a way for cats trying to cope with stressors by avoid them, hiding enrichment should be offered to each individual.

The limitations of the present study should be recognized when extrapolating any results. One of them is the relatively small sample size which may be not appropriate to represent an entire population. A larger sample size could give more representative outcomes and results. Although similar housing conditions are used as in previous studies cat population in certain area can differ from other regions and differences in management between shelters could influence outcomes (54,68).

CONCLUSION

In order to reduce stress it is important to offer cats adequate tools to cope with stressors in animal shelters, making them better capable to adapt to their new environment. Results suggest that offering hiding enrichment could help coping with the new environment by reducing physiological stress. Although, inter-animal variability is high and hiding boxes may not reduce stress in all cats, at least the option of hiding and perching should be offered. No significant differences were observed between the experimental group and the control group regarding to bodyweight loss, food intake, water intake, fURI score and adoption rates but descriptive statistics suggests the importance of a hiding box on improving adaptation, reflected by less mean decreases in bodyweights for cats in the experimental group. Determining body weights could therefore act as a relatively easy and practical way for recognizing impaired welfare. This study highlights the importance of hiding enrichment and applying this in practice will be an important step in increasing welfare.

Future research is necessary to define the benefits of offering a hiding box in single housing in quarantine area on reducing stress levels in subsequent social housing.

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