The Effect of a Diet Supplemented with Alpha-Casozepine and L-Tryptophan on Stress Levels in Dutch Shelter Cats

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Abstract

In 2016 the Dutch Society for the Protection of Animals took in 13.650 stray and relinquished cats with an average Length of stay (LOS) of 50 days. Cats in animal shelters are exposed to acute and chronic stress, which has implications for the animals welfare and health. Immunodeficiency increases the risk of developing several diseases.
Research has shown hiding enrichment to be an effective way in decreasing stress levels and thereby improving welfare of shelter cats. Effectivity of the supplements alpha-casozepine and L-tryptophan in reducing stress in cats have been investigated in household situations and showed promising results. The aim of this study was to determine if a diet supplemented with alpha-casozepine and L-tryptophan would reduce stress levels in shelter cats during the 14 day quarantine period. Place preference, feline upper respiratory infection (fURI) scores, body weight, food and water intake and adoption rates were used as parameters of stress.
9 Newly brought in European short or long hair cats between 1 and 10 years of age, were randomly divided into either the control group (N = 4), who were fed Royal Canin SC 365D® or the experimental group (N = 5), who were fed the supplemented diet (Royal Canin Veterinary Diet® - Calm™ cat). Place preference and fURI scores were observed on day 1, 2, 3, 5, 7, 9, 12 and 14. Body weights were determined on day 1 (week 0), day 7 (week 1) and day 14 (week 2). Food and water intake was registered every day for each cat. Length of stay (LOS) was defined as the number of days between leaving the quarantine room and being adopted and was noted for each cat.

Most important findings were:
1.Variations between individual animals were high especially in terms of place preference. Some cats spend almost the entire 160 minutes observation time in one place preference location.
2. Cats in both research groups spent most of the time in their hiding box. No significant difference was found between the control group and the experimental group, which indicated the supplemented diet not having any effect on place preference.
3. Sneezing and ocular discharge received the highest scores in fURI scoring. The scores for all fURI signs did not differ between the control and the experimental group, but a significant correlation was found between fURI score “sneezing” and quarantine room.
4. Loss of body weight during the 14 day observations period was not significant for all cats in both groups (body weight on day 14 compared to body weight on day 1).
5. A negative correlation was found between time spend in the hiding box and food intake.
6. Food and water intake for all cats in both research groups was significantly lower than the daily requirements. No significant difference was found for food and water intake between the control and the experimental group.

Previous studies show an effect of hiding enrichment on stress levels in shelter cats. The results of the present study indicate that a diet supplemented with alpha-casozepine and L-tryptophan does not have an effect on stress levels in shelter cats. The supplemented diet did not influence hiding frequencies neither did it prevent cats to develop fURI signs. Body weight losses as well as food and water intake did not differ significantly between the experimental group and the control group. This study shows the importance of monitoring food and water intake of cats housed in animal shelters.

Preface

This thesis was written as the final part of my Master of Veterinary Medicine at the University of Utrecht. Cats have always fascinated me, therefore I was really pleased I was able to do a research project concerning cats and their behaviour.

I would like to thank drs. W.J.R. van der Leij, dr. R.J. Corbee and Dr. C.M. Vinke for offering me this chance, for their enthusiasm and for their guidance and support.
Of course, huge appreciation and thanks to the animal shelter “Dierentehuis Stevenshage” for their help and hospitality. I have really enjoyed my time working there and could not have finished this project without their help.
Furthermore I would like to thank the Dutch Society for the Protection of Animals for providing the hiding boxes.

Thanks to Lara Selman for helping me out with all kinds of questions, to Jorik Vahl for supporting me during this entire project, for giving feedback and for helping me with the camera equipment and to Erwin van Eyk for helping me with the English language.

I also would like to thank all the people I have not mentioned, but contributed to this study or supported me in some way.

Daniëlle van Eyk
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Introduction

With a total of 2,6 million cats in the Netherlands, this feline companion is quite popular[1]. Despite this popularity, neglect and relinquishment is occurring frequently with various kind of reasons[2]. In 2016 the Dutch Society for the Protection of Animals took in 13.650 stray and relinquished cats with an average Length of stay (LOS) of 50 days[3]. Cats in animal shelters are exposed to acute and chronic stress depending on the time span in the shelter, which has implications for the animals welfare. Last year, research has been done to determine the effects of stress and how to minimalize the amount of stress in shelter cats[4-9]. One of these studies looked at weight loss and food intake as an effect of stress in shelter cats[7].

Acute stress factors are common within animal shelters: contact with unfamiliar cats and people, loud noises (most often caused by barking dogs), the loss of their own territory and a completely different environment can evoke a stress response in cats[10,11]. Stress is a biological response on a challenge to an individual's allostasis. Yet it is not by definition detrimental[12]. In a challenging situation a cat will express a combination of behavioural, autonomic and neuroendocrine responses. Examples of behavioural responses in answer to a challenging situation are running away or hiding from the situation. However these type of responses are not possible for cats in confinement. As a consequence cats could use their litterbox as a hiding substitute instead[8,12]. The autonomic nervous system response, which is the basis for the “fight or flight” response, will lead to signs of acute stress, .e.g. dilated pupils, flattened ears, tail close to the body and fully opened eyes[12,13]. This acute response involves hormones as norepinephrine and epinephrine and helps the individual to prepare for an adequate reaction to the challenging environment. If failing chronically to adequately react, the welfare of the individual is at stake[11,12,14].
A cat exposed to continuous stress or multiple acute stressors, without being able to adapt to these stressors, will experience chronic stress, which is characterized by the production of glucocorticoids like cortisol[15,16]. Chronic elevation of glucocorticoids has a suppressing effect on the cellular and humoral immune system leading to an increased risk for infectious diseases like upper respiratory tract infections[11,15,17].

Acute and chronic stress may lead to behavioural anomalies such as defecation outside the litterbox, over-grooming (potentially leading to alopecia) or decreased grooming[14,18]. It also increases hiding behaviour, decreases the elimination of faeces and urine and reduces the intake of food and water[7,16,19]. A decreased intake of food or in severe cases anorexia causes loss of body weight. Tanaka et al. (2012) showed 82% of the 60 individually housed cats losing body weight during their stay in a shelter. A quarter of all cats from this study lost more than 10% of its body weight[7]. Selman et al. (2017) found a 6,34% loss of bodyweight in cats provided with a hiding box[20].

Besides behavioural reactions of maladaptation, Feline Upper Respiratory tract Infections (fURI) are common in animal shelters, with feline herpesvirus (FHV) and feline calicivirus (FCV) as its primary pathogens. Other, less common, pathogens are *Mycoplasma, Chlamydophila felis* and *Bordetella bronchiseptica*[15,21]. Most cats develop a persistent infection with FHV after first exposure, with the trigeminal ganglia as the site of viral latency from where reactivation of FHV can occur. Stress can facilitate this reactivation, leading to fURI signs, within 11 days[7,22]. In contrast, FCV carriers shed more continuously[21,23,24]. Signs commonly seen in cats with fURI include serous or mucopurulent nasal and ocular discharge,

sneezing, conjunctivitis, inappetence and fever. In severe cases dyspnoea and coughing can also develop. Oral ulcerations are typically seen in infections with FCV and may cause excessive salivation[7,15,25]. fURI can be a challenge for animal shelters as vaccinations against these viruses do not prevent infection entirely. Additionally, transmission takes place easily through conjunctival or nasal discharge, direct contact and fomites[15,26]. Outbreaks of fURI in animal shelters occur frequently because of these factors, in combination with a large population of susceptible cats[7,26]. Outbreaks of fURI cause suffering for the cat, require extra personnel, are a financial burden for the shelter and extend the length of stay in a shelter[21,23]. Hence it is important to prevent the development of fURI by minimalizing stress in cats as much as possible.

A hiding box as environmental enrichment has already been proven to be an efficient way to reduce stress in shelter cats[6,8]. Hiding seems to be an important way for cats to handle stressful events by allowing them to express cat specific behaviour. Vinke et al. (2014) showed the significant effect of a hiding box on stress levels in shelter cats by using the Kessler and Turner Cat Stress Score (CSS)[6]. Stress scores decreased faster in cats provided with a hiding box while housed in a shelter compared to cats without hiding enrichment[6]. Although the CSS is a reliable method to measure stress in cats, for shelter staff it is time-consuming. Additionally it requires training and knowledge which makes the CSS unpractical for applying in a shelter setting. In this study we will therefore use place preference to determine the preference for a place anywhere in the cage. In the study of Vinke et al. (2014) cats without a hiding box spend 45% of the total observed time in their litterboxes in an attempt to get some covering, which is known as replacement hiding. Cats with a hiding box spend more than half the total observation time (55%) in their hiding box[6].
Cats also prefer elevated platforms as this vantage point enables them to better observe their surroundings and watch people and animals approaching[6,14,20,27]. Furthermore, cats show less stress when caretaking is predictable and consistent, thus it should be pursued to let daily care be performed by the same persons and around the same time[14,20,28].

In addition to environmental enrichment there are dietary options. Anxiolytic supplements are available claiming to reduce anxiety and stress in cats, but there are not many published studies about these supplements yet[29,30]. A commercial diet supplemented with alpha-casozepine and L-tryptophan is available for cats as a calming diet. Alpha-casozepine is a milk protein hydrolysate originating from Alpha-S1 casein, an important protein in cow milk[29]. This hydrolysate has an affinity for gamma-aminobutyric acid (GABA) receptors, which play an important role in the pharmacology, neurochemistry and physiopathology of stress and anxiety[31,32]. Alpha-S1 casein shows anxiolytic effects comparable to benzodiazepines in humans[33], rats[31], dogs[34] and cats[29] but less potent and without it's negative side effects. L-tryptophan is an amino acid and is primarily metabolized through the serotonin and kynurenine pathways. Both pathways are associated with anxiety and stress responses[35,36]. The increased intake of tryptophan leads to less irritable behaviour in healthy humans and has an anxiolytic effect in rats[37,38].

Studies found promising results of these supplements in cats that were staying in their caregivers homes[29,39]. Beata et al. (2007) showed anxious cats treated with alpha-casozepine (15mg/kg body weight/24hours) being less fearful for familiar and non-familiar people after 56 days of treatment, with an significant lower overall fearful behaviour score during

physical consultations. Though there was no significant difference in owner evaluation between the control and the treatment group in this study[29]. Miyaji et al. (2015) fed 10 physically and behaviourally healthy indoor cats a commercial diet (Royal Canin Veterinary Diet® Calm™ cat) supplemented with tryptophan (3,6g/kg) and alpha-casozepine (15g/kg) and 11 indoor cats with a control diet without alpha-casozepine and less tryptophan (3,4g/kg). Regarding acute stress the study diet showed no significant benefits. In contrast, after 8 weeks cats who were fed the supplemented diet had reduced urinary cortisol levels, whereas the control group showed no changes[39]. DeNapoli et al. (2000) found a decrease in anxiety-related behaviours in dogs after being fed a low-protein supplemented diet for one week[40]. This is due to the fact that tryptophan has the same blood-brain barrier transporter mechanism as other large neutral amino acids (tyrosine, phenylalanine, valine, leucine and isoleucine). A low-protein supplemented diet results in a higher ratio of tryptophan to these large neutral amino acids.
When socially to humans adjusted cats endure less stress or are capable to sufficiently cope with stress they are more friendly and extrovert. These behavioural traits positively influence the chance for adoption as most adopters consider the temperament to be important in choosing a cat[8,28,41]. Lower Length of Stay (LOS) will imply improvement of welfare, less costs for a shelter and a lower infection pressure of infectious diseases in a shelter.

Several studies investigated different kinds of solutions to minimalize stress levels in cats whilst staying in their caregivers homes, including supplements as alpha-casozepine and tryptophan. However, the applicability of a diet supplemented with alpha-casozepine and tryptophan diet in shelter cats was never studied.
Therefore, the purpose of this study was to evaluate the effect of a diet supplemented with alpha-casozepine and L-tryptophan (Royal Canin Veterinary Diet® Calm™ cat) on newly arrived cats during the 14 days in quarantine in Dutch animal shelters by determining place preference, body weight, food and water intake and development of fURI signs.
Considering the previous research, we expect cats fed the supplemented diet will lose less body weight, show lower fURI scores, have higher food and water intake and adapt themselves faster to the shelter environment than the control group. Adaptation to the quarantine room is reflected by spending less time in their hiding box, which will be controlled by periodic recording of place preference[6,20].

Adoption dates are registered and Length of Stay is defined as the number of days between leaving the quarantine room and getting adopted. As cats with lower stress scores will be more extrovert and friendly, we expect cats fed the experimental diet to have a shorter Length of Stay than the control group.
Previous studies already showed the beneficial effect of a hiding box on stress levels in shelter cats[6,8,20]. In this study we will provide all cats with a hiding box to determine if the effect of a supplemented diet is able to significantly reduce stress levels even more.

Material & Methods

Study site

All data were obtained at, "Dierentehuis Stevenshage", an animal shelter in Leiden (the Netherlands). This shelter took in and rehomed 520 cats in 2017. These cats were either strays (347), relinquished (99), exchanged (36), returned after placement (12) or born in the shelter (26), see Appendix 1. The quarantine section consists of 8 rooms with 6 quarantine cages per room, providing a total capacity of housing 48 cats[42].

Study subjects

Equal to the study of Vinke et al. (2014) and Selman et al. (2017) the following criteria were used to select cats for this study[6,20]:

- Breed: cats included were European domestic shorthair cats and longhair cats without a distinct breed.
- Age: only cats between 1 and 10 years of age were included. Besides having a higher incidence of medical conditions, older cats can also show behavioural changes. Over 25% of all cats aged 11 to 14 showed behavioural changes in a study by Gunn-Moore et al. (2007). Younger cats are not incorporated in this study as they are more adaptable and modifiable in their behaviour and more susceptible to stress-associated infections than adult cats[17,23,43,44].
- Gender: male and female cats, both castrated and non-castrated cats were used in this study as previous studies did not show significant differences between gender in ability to cope with stressors[8,9].
- Health status: upon arrival a physical examination was performed by a veterinarian. Only cats healthy upon arrival were incorporated in this study. Meaning they did not show any signs of illness or injury. Besides, only non-pregnant cats were used in this study.

In this study 9 newly arrived cats met the preceded criteria (Table 1, Appendix 2). The selected cats were randomly assigned to the experimental group or the control group, using a list randomizer[a]. The experimental group was offered a supplemented (L-tryptophan, alpha-casozepine) diet and the control group a non-supplemented diet. The control group consisted of 4 cats (all female). The experimental group consisted of 5 cats (1 male, 4 females). All cats were either stray or relinquished, cat 6 being the only cat castrated upon arrival. In both the control and the experimental group 3 female cats were castrated during the study period (on day 6). Fasting started at 22:00h the night before the castration. No food was offered in the morning on the day of the castration, their first meal being the afternoon meal on the day they returned from the veterinary clinic (day 6). Pain medication was only given during the castration, so no medication was provided in the days after castration unless there was a complication during or after the castration. Cat 4 developed a fever and did not want to eat after the castration, from day 7 to day 10 she therefore received meloxicam (0,05mg/kg/day).
Cat 11, 12 and 14 were longhair cats without a distinct breed, all other cats were European domestic shorthairs. Age in the control group ranged from 12 to 73 months with a mean age of 32 months (± 24 SD) and a median of 21 months. Age in the experimental group ranges from 15 to 73 months with a mean age of 47 months (± 20 SD) and a median of 48 months.

a: www.listrandomizer.org

As no exact birthdates were known for all cats, age was estimated by the veterinarian based on the body condition score and teeth. These ages could deviate from their biological ages.

	Control group					*Experimental group*				
Cat No.	*Gender*	*Age*	*Weight*	*Surrender type*	*Cat No.*	*Gender*	*Age*	*Weight*	*Surrender type*
3	F	73	3,05	R	4	F	73	2,83	R
5	F	12	2,99	S	6	F	48	2,94	S
11	F	28	2,68	R	7	M	36	6,45	S
13	F	16	3,05	R	12	F	64	3,20	R
					14	F	16	2,32	R

Table 1 Participating cats. Age in months, weight in kg. F = female, M = male, R = relinquished, S = stray. Cat 6 (female) in the experimental group was the only cat castrated upon arrival.

Housing conditions

The Dutch legislation states that stray cats entering an animal shelter should be kept for two weeks in holding, after which the shelter will receive ownership[45]. During these two weeks cats stay in a quarantine area, housed solitary. Although not obligatory, relinquished cats with an inadequate vaccination history also stay in the quarantine area for two weeks in this shelter. In this study all selected cats were followed during these 14 days in quarantine rooms. This way the same environment was established for all cats. Three quarantine rooms (B, G and H) were used, all of them the same size and containing the same cages. In each quarantine room there were six cages each the same size: 85 x 70 x 56 cm (L x W x H). Each cage contained an open litterbox, a food- and water bowl, a hiding box and a perching shelf at a height of 26,5 cm . A towel can influencing place preference because it is more comfortable than an uncovered floor. Four towels were therefore placed through the entire cage: on the floor of the cage, on top of the shelf and inside the hiding box (Fig 1.)

Fig. 1 Cage arrangement

Fig. 2 The hiding box

Cats from both the control group and the experimental group were placed randomly in one of the three quarantine rooms to reduce environmental influences.
Because cats do not take on a relaxed body posture in cold temperatures, the temperature was not allowed to drop below 15°C[9]. An automatic climate system regulates the

temperature and exact temperature was measured in all three quarantine rooms two times a day by the observer. Temperature in room G ranged from 21.1 to 25.5°C with a mean of 23.6°C (SD = 1.18), in room H from 21.4 to 25.0°C with a mean of 23.6°C (SD = 0.96) and in room B from 21.9°C to 24.3°C with a mean of 23.0°C (SD = 0.82). Daylight was provided by windows opposite to the cages and one fluorescent lamp per room provided light between 08:00h and 17:00h.

The hiding box provided to all cats in both study groups was placed underneath the perching shelf and was provided by the Dutch Society for the Protection of Animals (Fig 2. Fig. 2). These boxes were from the same batch as the boxes used in the studies by Bidlot et al. (2018) and Selman et al. (2017). Boxes were made out of white cardboard and measured 44 x 31 x 26 cm (L x W x H). One entrance was present on the front of the box (W x H: 16 x 21cm). To resemble the boxes from the previous studies, a second opening on the left side was manually made (W x H: 16 x 19cm). Boxes were not reused between cats.

Standard procedures

Each newly arrived cat was checked for the presence of a microchip. Stray cats without a chip or when no phone number was available were photographed to facilitate finding a possible owner on social media. Each Tuesday and Friday the shelter's veterinarian came in from 10:00h to 12:00h. Cats were examined, vaccinated and chipped if no microchip was present. The Dutch legislation states that cats with an unknown or incomplete vaccination status upon arrival in an animal shelter need to be vaccinated within 5 days against feline panleukopenia, feline herpesvirus and feline calicivirus[46]. In this animal shelter Felocell® CVR (Pfizer Animal Health) was used, which is a parenteral non-adjuvanted modified-live vaccine, containing attenuated strains of feline herpes virus, feline calicivirus, and feline panleukopenia virus. All cats with an unknown or incomplete vaccination status were vaccinated within 5 days upon arrival and boostered after two weeks. 7 days after receiving the second vaccination cats were allowed to transfer to the adoption floor. Cat 5, 6 and 7 were stray cats with an unknown vaccination status and were vaccinated on day 6, day 4 and day 3 respectively. Cat 3, 4, 11, 12, 13 and 14 were relinquished but had an incomplete vaccination status and were therefore vaccinated on day 2.
If necessary cats were treated for fleas and ticks. Castration of stray cats took place after the two week quarantine period. Relinquished cats were castrated during the quarantine period on day 6. Tomcats were castrated at the shelter, female cats were taken to the veterinary clinic and returned to the shelter the same day.
Two shelter employees took turns in cleaning the cages and daily caretaking between 8:00h and 12:00h. Spot-cleaning was applied, meaning only dirty spots in the cage were cleaned. The bottom of the cage was swiped, the litterbox was emptied, cleaned and refilled (Linda Hout kattenbakvulling®) and drinking water was refreshed. Towels were replaced when wet or soiled. Food was served twice a day: between 8.00h and 10.00h and between 16.00h and 17.00h. Cats usually remained in their cages during cleaning.
After the quarantine period the cages were cleaned thoroughly and disinfected before reusing.

Diets

The experimental diet used in this study was a diet supplemented with alpha-casozepine and L-tryptophan, Royal Canin Veterinary Diet® - Calm™ cat, whereas the control diet was the Royal Canin SC 365D®. The latter diet is a commonly used non-supplemented diet in shelter cats. Details of these two diets are shown in Table 2, Table 3 and Table 4. Diets were packed in non-labelled food containers, see Fig 3. These containers were coded diet "A" and diet "B". Diet A being the control diet (Royal Canin SC 365D®) and diet B the experimental diet (Royal Canin Veterinary Diet® - Calm™). Content of the diets were known only to the director of the animal shelter and one clinician of the University of Utrecht. They made sure the right diet ended up in the right food container. The observer and other clinicians involved in this study did not know which diet was the experimental diet and which diet was the control diet, making this a double-blinded controlled trial.

Fig. 3 Food container

Amounts of both diets for each cat were calculated based on their body weight on day 0. The following formulas were used:

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

DER (daily maintenance energy requirements) = 1.4 x RER

Royal Canin Veterinary Diet® – Calm™ cat $= \frac{\text{DER}}{3{,}434\text{kcal/gram}}$

Royal Canin SC 365D® = $\frac{\text{DER}}{4{,}066\text{kcal/gram}}$

Both formulas were named either formula A or B (corresponding with the labels on the food container) in Excel by the clinician of the University of Utrecht to whom the contents of both diet A and B was known. This way only body weights had to be filled in in either formula A or B to know the exact amount of required food for each cat. As metabolizable energy is given in kcal/kg for both diets (Table 2) these could be converted to kcal/gram.
Cats were fed quantities sufficient for underweight cats, calculated by using a factor 1.4 for the DER[47]. Thus all cats were offered 116% of their required daily food intake. These requirements were based on factor 1.2 (inactive neutered adult) for the DER[47]. Although cats in a cage do not have a lot of exercise, feeding inadequate amounts of calorie requirements was prevented to be a reason for loss of body weight.

	Royal Canin Veterinary Diet® - Calm™ cat	Royal Canin SC 365D®
Protein	360	300
Fat	110	190
Crude fiber	40	31
Crude ash	79	75
Moisture (%)	5,5	5,5
Metabolizable energy (kcal/kg)	3434	4066
Alpha-casozepine	0,94	0
Tryptophan	3,6	NA

Table 2 In g/kg DM (dry matter) unless stated otherwise, NA = not available

Royal Canin Feline Calm Diet®
Chicken by-product meal, corn, brewers rice, wheat gluten, corn gluten meal, wheat, natural flavors, powdered cellulose, dried plain beet pulp, chicken fat, fish oil, calcium sulphate, salt, potassium chloride, DL-methionine, vegetable oil, fructooligosaccharides, taurine, L-lysine, psyllium seed husk, choline chloride, vitamins, dried hydrolyzed casein, L-tryptophan, marigold extract (Tagetes erecta L.), trace minerals [zinc proteinate, zinc oxide, ferrous sulphate, manganese proteinate, manganous oxide, copper sulphate, calcium iodate, sodium selenite, copper proteinate], L-carnitine, rosemary extract, preserved with mixed tocopherols and citric acid.

Table 3 Ingredients of the experimental diet

Royal Canin SC 365D®
Cereals, meat and animal derivatives, oils and fats, derivatives of vegetable origin, vegetable protein extracts, minerals, yeasts

Table 4 Ingredients of the control diet

Experimental set up

To facilitate this experiment, only two shelter employees were allowed to enter the rooms with cats participating in this study. Other employees and volunteers were informed, but not involved in this study.
Upon arrival of the cat one of the employees examined the animal, checked if a microchip was present and registered their gender. A few days later, depending on the day of arrival the shelters veterinarian registered their health status and age, which was estimated if no date of birth was known. Body weight was measured by the observer, as were fURI scores, place preference, food- and water intake and adoption rates.

Data collection took place between 12:00h and 16:00h, after daily cleaning and between the morning and afternoon feeding time. Thereby minimalizing influence from employees in the rooms or people walking across the hallway.
For behavioural assessment cats were observed using a video camera (Bascom®DVR). The observer was situated in another room outside the quarantine area where a computer screen was connected to the video camera (Fig. 4, Fig. 5 and Fig. 6). This way any influence

from the observer on the cats' behaviour was prevented. The camera was placed in front of the cage in a position enabling a total overview of the cage on screen. When the cage was not completely visible the position of the camera was adjusted. After positioning the camera, each cat was given 2 minutes to adjust to the camera equipment and to recover from the temporary presence of the observer. If, during the 20 minute behavioural assessment, some kind of interruption occurred (someone walking across the hallway, loud sounds), recording was stopped and restarted after another 2 minutes of adjusting time. fURI signs were scored after the behavioural assessment to prevent influence of contact with the observer. All observations were carried out by one observer.

Fig. 4 Camera positioning

Fig. 5 Equipment set up for behavioural assessment

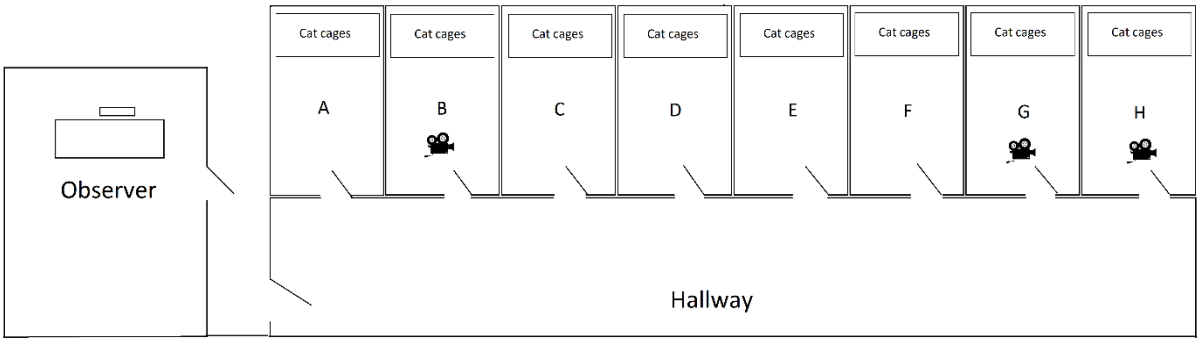

Fig. 6 Experimental set up in the shelter

Data collection

Data were collected between July 23 and September 16, 2018

Place Preference

Place preference was scored for 20 minutes on day 1, 2, 3, 5, 7, 9, 12 and 14 (160 minutes total) during the behavioural assessment. To achieve the most accurate way of scoring behaviour, an All Occurrences Sampling method was used according to the study of Vinke et al. (2014)[6]. This means that the duration and the frequencies of specific behaviour were observed through continuous recording.
The following situations were distinguished in place preference:

- On the shelf (situation 1)
- In the hiding box (situation 2)
- Elsewhere hiding
    - In the litterbox (situation 3a)
    - Behind the litterbox (situation 3b)
- Elsewhere non-hiding (situation 4)

The criteria of a situation is met when "the animal has more than two paws or more than 50% of its total body weight in that part of the cage"[6,20]. Each situation was registered and expressed as a fraction of the total of 20 minutes to acquire a daily place preference per cat. These fractions were averaged per research group providing mean daily scores. Finally, the mean place preference for the total observation period (160minutes) was determined for both the control and the experimental group. The purpose for this differentiation was to see if an supplemented diet would alter hiding activity.

fURI Index

In the study of Selman et al. (2017) a modification of the scoring method Bannasch and Foley was used to assess severity of fURI. None of the cats developed fURI signs in that study which could be caused by an insensitive scoring method. Therefore the scoring method of Litster et al. (2015) was used in this study, which was converted to an observation period of 20 minutes corresponding with the study by Bidlot et al. (2018), Fig. 7[48,49]. The definition of fURI as described by Dinnage et al. (2009) was used: "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"[50]. Each cat was rated 0, 1, 2 or 3 for each clinical sign depending on severity. fURI signs were scored after scoring place preference on day 1, 2, 3, 5, 7, 9, 12 and 14. Sneezing and coughing could be seen during the behavioural assessment of 20 minutes.

Scores were accumulated per cat per day for each clinical sign. A mean daily severity score per group was determined per clinical sign as well as a mean severity score for each clinical sign for the total observation time for both groups.

Clinical signs	Score 0	Score 1	Score 2	Score 3
Ocular discharge	No	Small amount of serous discharge	Large amount of serous discharge	Mucopurulent discharge
Nasal discharge	No	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	No	Sneeze 1 time/20 min	Sneezes 2-3 times/20 min.	Sneezes 4 or more times/20 min.
Coughing	No	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. 7 Scoring method for severity of fURI, from the study by Litster et al. (2015), converted to an observation period of 20 minutes[48,49].

Body weight

Body weighs were measured by the observer using an electronic scale with a maximum of 30kg and an accuracy of 10gram. On the day of arrival (day 0) body weights were registered to calculate the exact amount of food for each cat. Subsequently, body weights were registered on day 1 (week 0), day 7 (week 1) and day 14 (week 2). To prevent interfering with the behavioural assessment, cats were weighed early in the morning after feeding and daily cleaning.
Individual body weights were noted and averaged per study group per day and for the total observation time. Weight losses and gains were registered as a percentage of the initial body weight for each cat and averaged for both research groups.

The following ranking according to Tanaka et al. (2012) was used to classify weight losses[7].

1. No weight loss
2. 0.1 – 4.9% body weight loss
3. 5-10% body weight loss
4. > 10% body weight loss

Food and water intake

Cats were fed their daily required amount of food equally divided over two feeding times, one in the morning and one in the afternoon. At feeding time, the food bowl was removed from the cage and the amount of remaining kibbles was registered on a form for each cat (Appendix 3). As food was offered twice a day, leftover food was registered in the morning and afternoon. Weight of the left over kibbles was registered using a kitchen scale with a maximum of 5kg and an accuracy of 1gram. After registering, the left over kibbles were thrown away, the bowl was cleaned and refilled with the right amount of food. Food intake was calculated by deducting the leftover kibbles from the offered amount of kibbles.

Water bowls were either filled with 200 or 250militers of drinking water. Once a day, in the morning, left over water was measured using a measuring cup and registered on the same form as mentioned above. Leftover water was thrown away, the bowl was cleaned and then refilled. At feeding time in the afternoon, water bowls were checked. When water bowls were empty or nearly empty they were refilled again and this was registered on the form (Appendix 3).
Food and water intake was averaged for the experimental group and the control group per day and for the total 14 days observation time.

Length of Stay

Adoption dates were noted to determine the Length of Stay (LOS). Length of stay was defined as the numbers of days between leaving the quarantine room and the adoption date. The LOS was used to determine if cats fed a diet supplemented with alpha-casozepine and L-tryptophan would be adopted sooner. The average length of stay was determined for both the experimental and the control group.

Statistical analyses

A randomised controlled trial (RCT) design was used for this study. Data were stored in two Microsoft Excel 2016 files (Microsoft Corp, Redmond, Washington), one file for the control diet and one file for the experimental diet. Statistical analyses were performed with the statistical program IBM SPSS Statistics version 25.0 (IBM Corp, Amronk, NY).

To test whether data (mean frequencies of each place preference location, fURI scores, body weights, weight losses, food and water intake, food : water ratio's, and length of stay) were normally distributed, a Shapiro-Wilk tests was used, which has a high power for asymmetric and symmetric distributed data and is appropriate for small sample sizes ($N < 50$)[51]. P values of less than 0.05 were regarded as statistically significant. Meaning; when $p < 0.05$, data was considered not normally distributed. Besides the Shapiro-Wilk test a visual inspection of the boxplots was carried out to determine the distribution of the data and to detect possible outliers.

The homogeneity of variance of the normally distributed data (body weight loss, length of stay) was verified by a Levene's test. A value of $p < 0.05$ was considered statistically significant, implying a difference in the variances. An independent-means t-test was executed to detect any differences between the control group and the experimental group for mean body weight loss and mean adoption rates.
A non-parametric test was performed when data had a non-normal distribution. Mann-Whitney U tests were executed to determine significant differences between the control group and the experimental group for mean frequencies of each place preference location, mean body weight and mean food and water intake. P values of less than 0.05 were regarded as statistically significant for both the Mann-Whitney U tests and the independent-means t-tests.

A linear mixed model was carried out to test for correlations between bodyweight, time, gender, age, surrender type and treatment group. Treatment group, time, gender and

surrender type were set as fixed factors, age as covariate and cat as subject. A linear mixed model was also used to test for correlations between food and water intake and treatment group, gender, surrender type and weight. And a linear mixed model was used as well to detect any correlations between Length of Stay and gender, age, surrender type or treatment group.

A related samples Wilcoxon signed-rank test was used to test for differences within-groups for place preference, fURI scores, body weight and food and water intake. To detect any significant differences between the three quarantine rooms for place preference and fURI scores a Kruskal-Wallis H-test was used. In both test, a P value of less than 0.05 was considered significant.

Results

Place Preference

A Shapiro-Wilk test was used to test the normality of the mean frequencies of each place preference location for both the control and the experimental group. In the control group, normality was found for the situations “perching shelf” ($D(4) = 0.999$, $p = 0.996$), “hiding box” ($D(4) = 0.863$, $p = 0.271$), “behind litterbox” ($D(4) = 0.782$, $p = 0.074$) and “elsewhere” ($D(4) = 0.976$, $p = 0.880$). In the experimental group data was normally distributed for the situations “hiding box”($D(5) = 0.873$, $p = 0.277$) and “elsewhere” ($D(5) = 0.900$, $p = 0.408$). A non-normal distribution was found in the experimental group for the situation “perching shelf” ($D(5) = 0.735$, $p = 0.021$).
No cats were observed sitting in their litterbox during the entire observation 14 days observation period in both groups (Appendix 4).

Differences between the control group (diet A) and the experimental group (diet B) for each place preference location were tested for significance with the Mann-Whitney U test (Fig. 8). No significant differences were found between both groups for the mean place frequencies of each location; perching shelf (control group: 32%; experimental group: 18% ; U = 6.00; $p > 0.05$), hiding box (control group: 40%; experimental group: 72% ; U = 5.00; $p > 0.05$), behind litterbox (control group: 8%; experimental group: 0% ; U = 5.00l; $p > 0.05$), elsewhere (control group: 20%; experimental group: 10%; U = 6.5; $p > 0.05$).

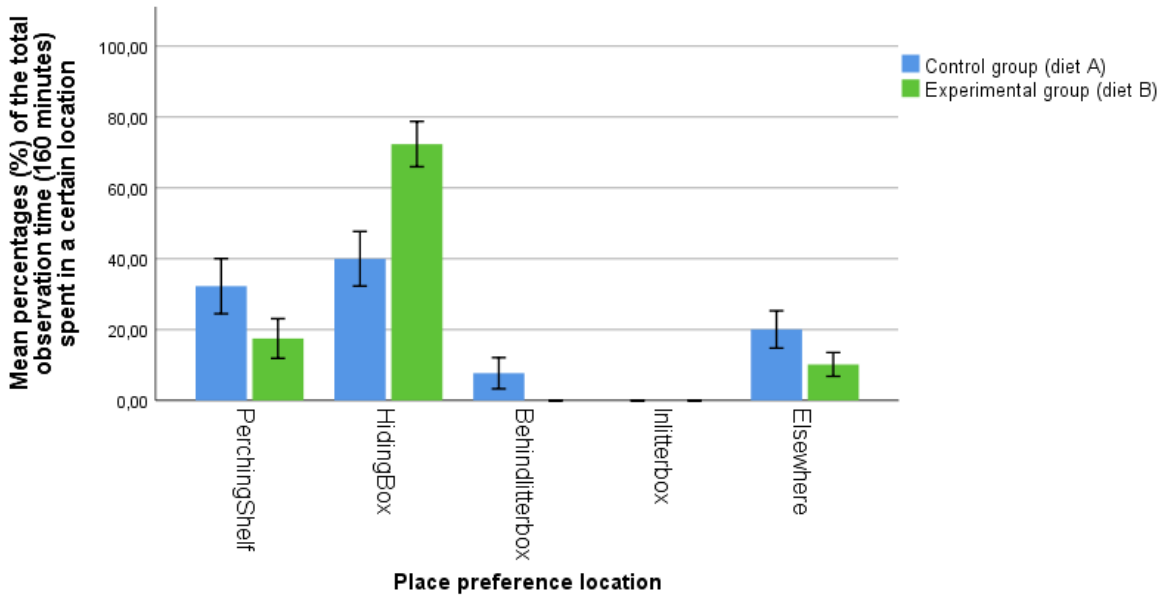

Fig. 8 Mean frequencies of the place preference locations of the total observation period (160minutes) in the control group (N=4) and the experimental group (N=5). Error bars represent ± SEM, differences between both groups are not significant.

In the control group cats were observed, during the 20 minutes observation time, hiding behind their litterbox on day 9 (30%), 12 (7%) and 14 (25%). Cats in the experimental group did not hide behind their litterbox, though these differences between group A and B were not statistically significant (U = 5.00; U = 7.5; U = 7.5; $p > 0.05$). As seen in Fig. 9 and Fig. 10

time spend in each location differed in time in both groups. On day 5 time spend in the hiding box decreased in the control group. Contrarily, cats in the experimental group spend most of their time in the hiding box during the entire 14 day observation period.

Time spend in the hiding box in the experimental group was at its highest on day 7 (97%). Cats in the control group spend 9% of their time in the hiding box on that day which differed significantly from the experimental group (U = 0.0; p = 0.016). A significant difference between both groups was also found for the location perching shelf on day 7 (U = 2.5; p = 0.048).
No differences were found for each place preference location ("perching shelf", "hiding box", "behind litterbox", "elsewhere") between the observation days, using a related samples Wilcoxon signed-rank test.

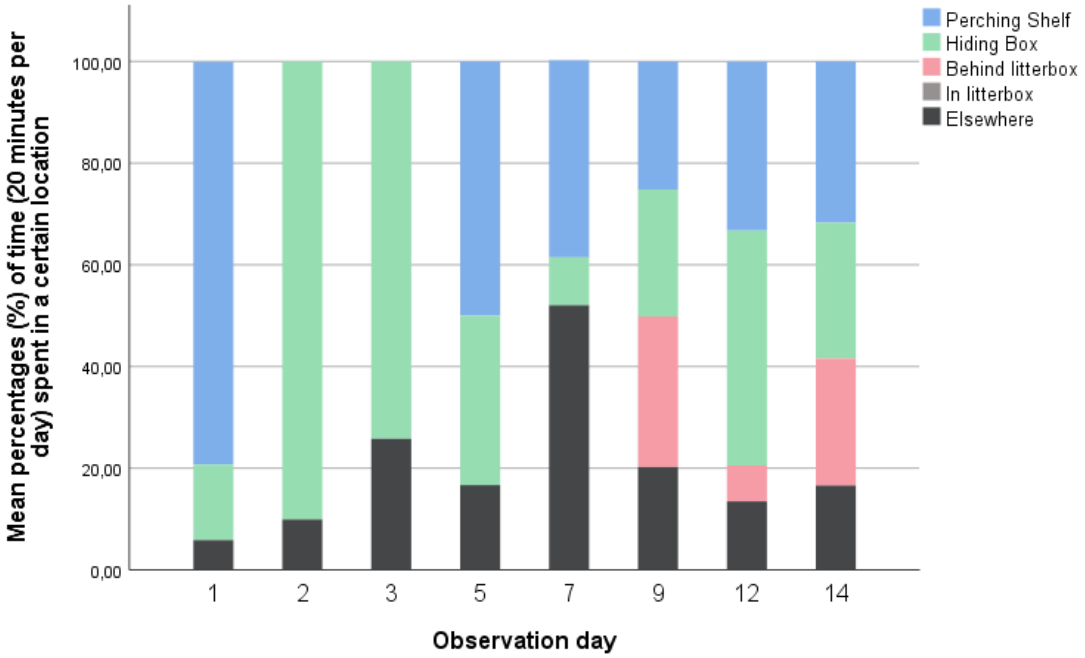

Fig. 9 Mean frequencies of the place preference locations in percentages of the observation time (20minutes) in the control group (N=4, diet A) on observation days 1, 2, 3, 5, 7, 9, 12 and 14.

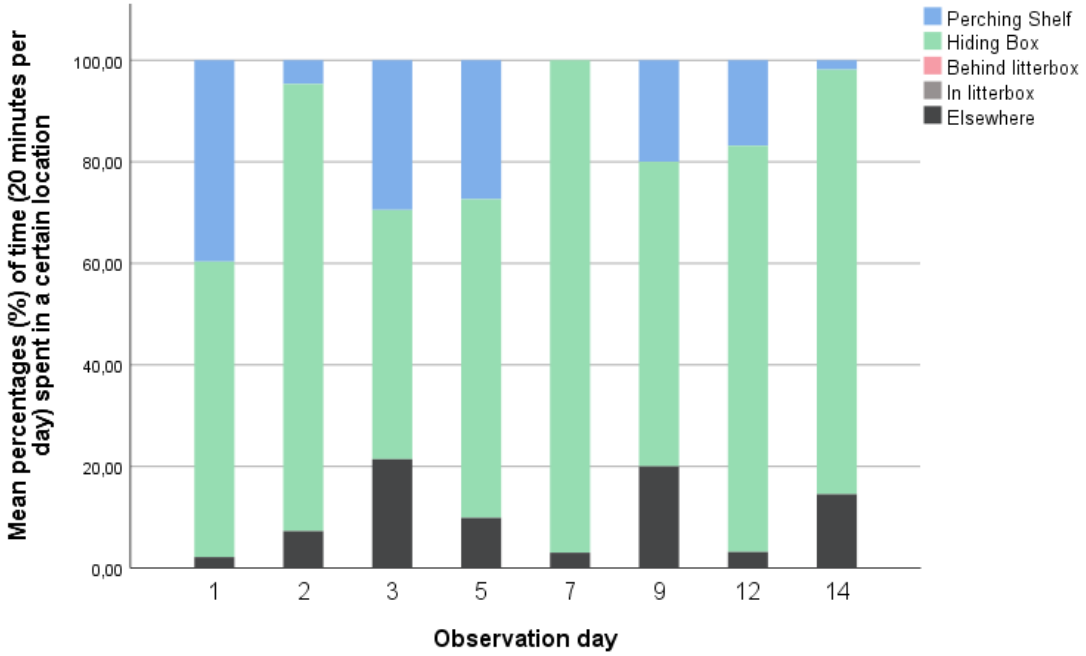

Fig. 10 Mean frequencies of the place preference locations in percentages of the observation time (20minutes) in the experimental group (N=5, diet B) on observation days 1, 2, 3, 5, 7, 9, 12 and 14.

Place preference was determined per cat to determine variance within groups. Boxplots did not show any outliers, though differences between individual cats were extensive (Fig. 11, Fig. 12).

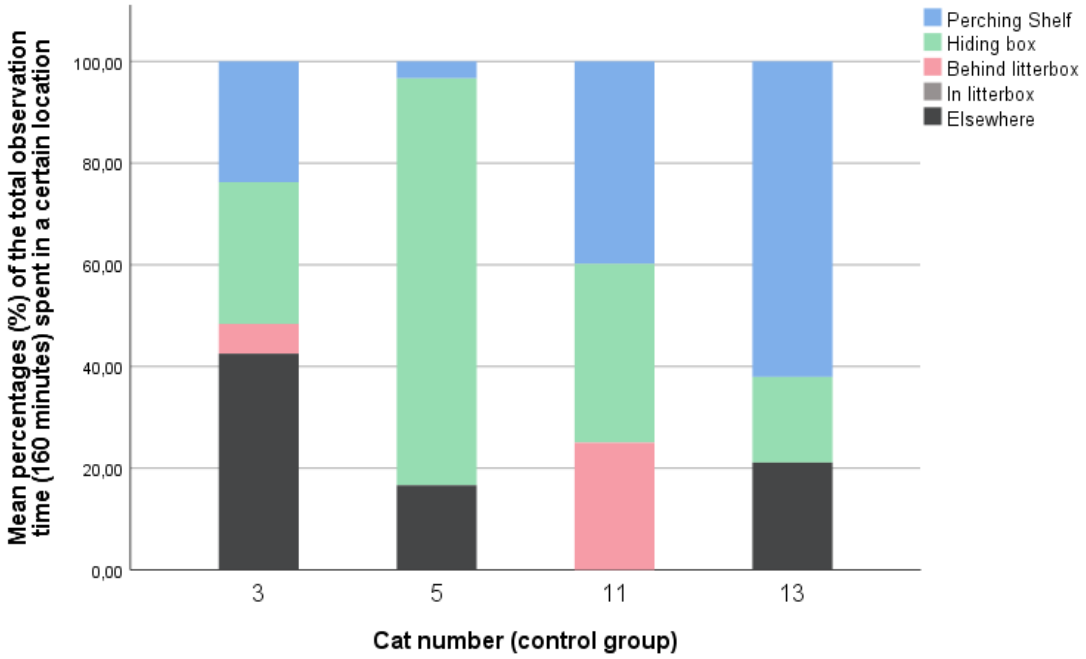

Fig. 11 Mean frequencies of the place preference locations in percentages of the total observation time (160 minutes) in the control group per cat (N=4).

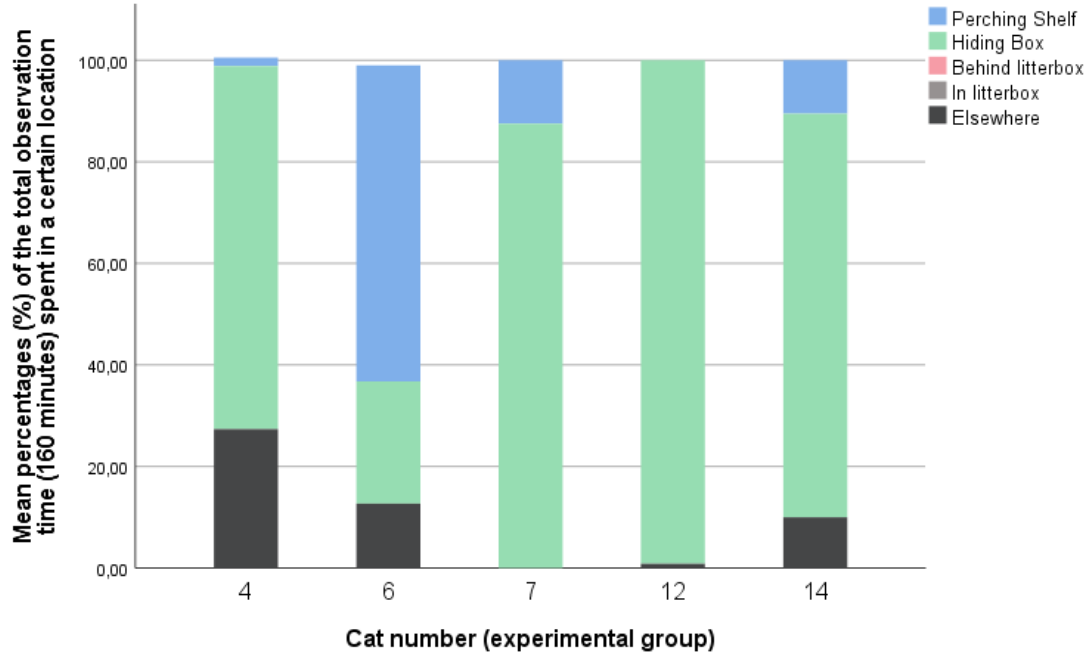

Fig. 12 Mean frequencies of the place preference locations in percentages of the total observation time (160 minutes) in the experimental group per cat (N=5).

In the control group, cat 5 was observed in the hiding box more often than other cats from this group (80%), while cat 13 spend a notable time on the perching shelf (62%). Cat 3 and 11 were seen hiding behind their litterbox for respectively 6% and 25% of the total observation time and did not show a preference for a specific location. In the experimental group cat 7, 12 and 14 spent more than the average 72% of the total observation time in the hiding box. Cat 4 and 6 spent less than the average time in the hiding box, whilst cat 6 preferred the perching shelf.
Overall, cat 13 (control group) spent the shortest amount of time in the hiding box (17%) and cat 12 (experimental group) the longest amount of time (100%). Cat 3 (control group) spend the longest amount of time elsewhere (43%).
To determine if differences in the quarantine rooms had any influence on the data, place preference was determined for each quarantine room (B, G and H), see Fig. 13. One cat from the control group (cat 3) and two cats from the experimental group (cat 4 and 7) were situated in room G. Cat 5 (control group) and cat 6 (experimental group) were situated in room H and cat 11 and 13 (control group) and 12 and 14 (experimental group) were situated in room B. In all rooms the most time was spent in the hiding box (B: 58%, G: 41%, H: 52%). Cats in room G spent more time elsewhere (28%) than cats in room B and H. Cats hiding behind their litterbox were only seen in quarantine room B (6%) and G (2%).
Kruskal-Wallis tests were performed comparing frequencies for each location between the three rooms. No significant differences were found between the rooms for each place preference location ($p > 0.05$).

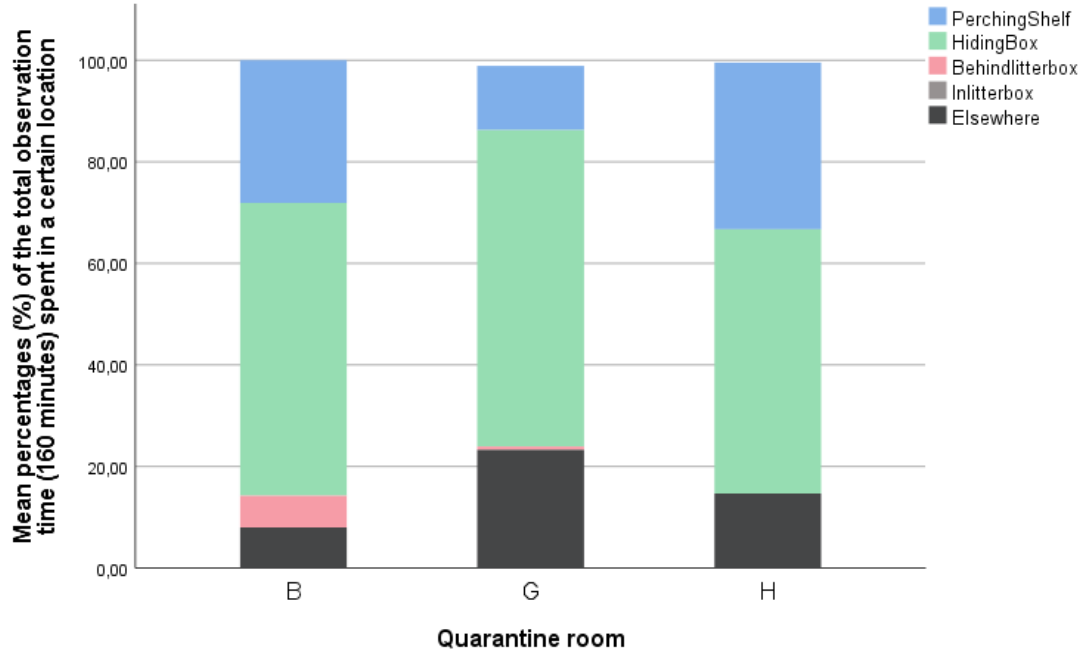

Fig. 13 Mean frequencies of the place preference locations of the total observation time (160minutes) in quarantine room B (N=4), G (N=3) and H (N=2).

Feline Upper Respiratory Infection (fURI) Scores

Cat 6 was healthy upon intake but developed ocular discharge on the first day, all other cats had a fURI score of 0 for all clinical signs on day 1 (Appendix 5). Cat 3 did not develop any fURI signs during the entire 14 days observation period. Clinical signs seen in all other cats were ocular discharge, sneezing, respiration and nasal discharge (Table 5). Coughing or an altered demeanor was not seen in any of the cats during the study period. At day 3, 3 out of 4 cats from the control group and 3 out of 5 from the experimental group had developed one or more fURI signs. At day 7 all cats, except cat 3, had developed one or more fURI signs.

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 12	Day 14
Diet A								
Ocular discharge	0	0.25	0.75	0.75	0.75	0.75	0.75	0.75
Nasal discharge	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0
Sneezing	0	0	0	0.25	0.75	0.75	1.25	1
Coughing	0	0	0	0	0	0	0	0
Demeanor	0	0	0	0	0	0	0	0
Diet B								
Ocular discharge	0.2	0.4	0.6	0.8	0.8	0.8	1	0.8
Nasal discharge	0	0	0	0	0.2	0.2	0.4	0.2
Respiration	0	0	0	0	0.2	0.2	0	0
Sneezing	0	0	0	0	0	0.2	0.6	0.4
Coughing	0	0	0	0	0	0	0	0
Demeanor	0	0	0	0	0	0	0	0

Table 5 Mean fURI scores per day per clinical sign for the control group (N=4) and the experimental group (N=5).

Ocular discharge was most frequently seen, 3 out of 4 cats from the control group and 4 out of 5 cats from the experimental group developed ocular discharge. On day 3, mean ocular discharge scores did not increase till day 14 for the control group, whereas an increase was seen till day 5 and again on day 12 in the experimental group (Table 5, Fig. 14). A Shapiro-Wilk test showed non-normal distributions for all observations days. A Mann-whitney U-test showed no significant differences between the experimental and the control group (U = 9.00, z = -0.251, *p* = 0.802) concerning ocular discharge for the overall mean scores and the score per day. Median score for the 14 day observation period for fURI score "ocular discharge" was 1.00 (IQR = 1.00) for the control group and 1.00 (IQR = 1.00) for the experimental group.
Sneezing was seen in 2 out of 5 cats from the experimental group and in 3 out of 4 cats in the control group starting at day 5 (Table 5, Fig. 15). According to the Shapiro-Wilk test, data was non-normally distributed for all observation days. Median score for the total observation period was 0.00 (IQR = 1.00) for the control group and 0.00 (IQR = 0.00) for the experimental group. Boxplots showed cat 12 (day 12) and cat 14 (day 9, 12 and 14) being outliers in the experimental group. Between both groups no significant differences were found between mean scores of the total 14 day observation period and score per day using a Mann-whitney U-test (U = 4.50, z = -1.414, *p* = 0.157).
Nasal discharge and respiration signs were only seen in cat 7 (experimental group) starting at day 7, with nasal discharge receiving the highest score on day 12. This cat did not develop any other fURI signs during the 14 day observation period.

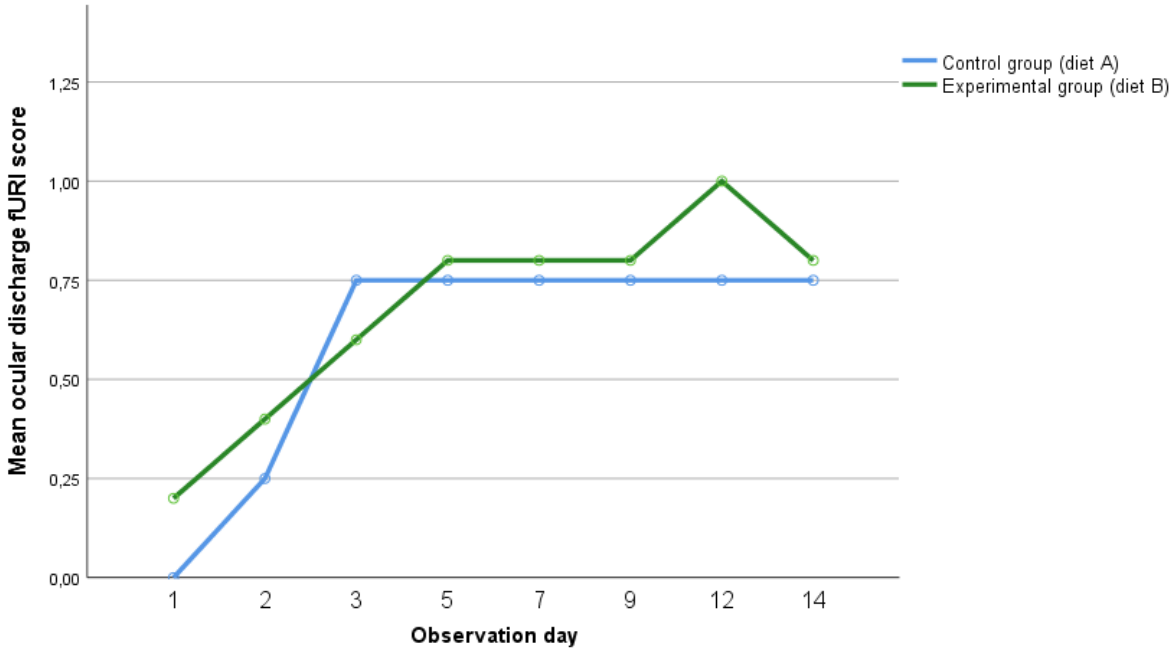

Fig. 14 Mean fURI score for the clinical sign "ocular discharge" for the control group (N=4) and the experimental group (N=5) on observation days 1, 2, 3, 5, 7, 9, 12 and 14.

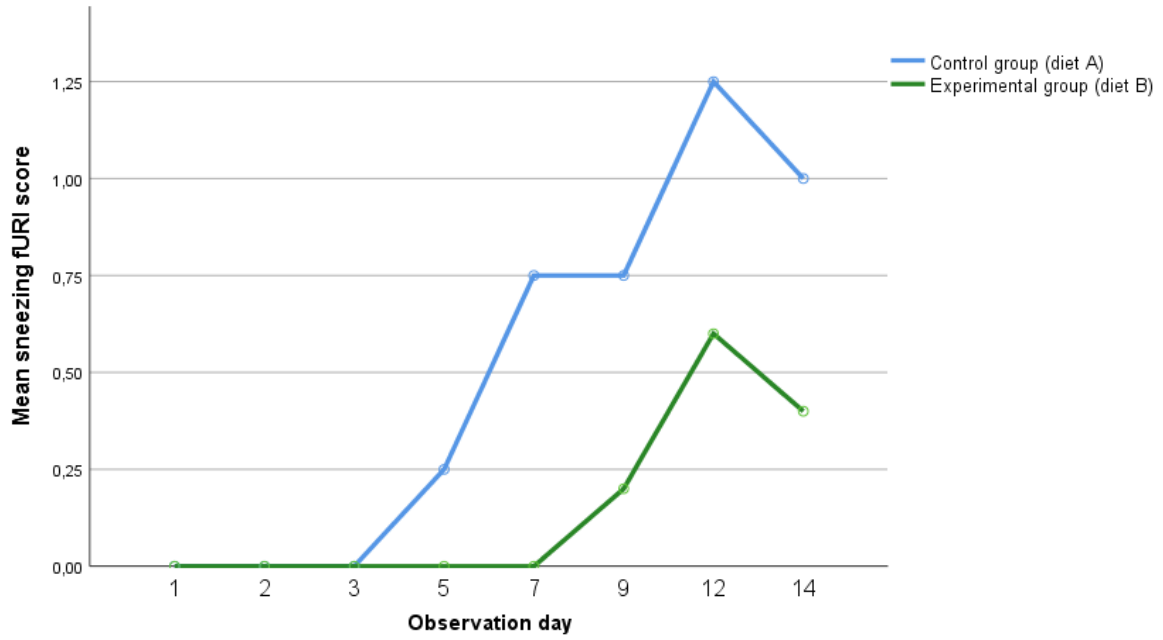

Fig. 15 Mean fURI scores for the clinical sign "sneezing" for the control group (N=4) and the experimental group (N=5) on observation days 1, 2, 3, 5, 7, 9, 12 and 14.

All cats received a vaccination (Felocell® CVR) during the 14 day observation period, on day 2 (cat 3, 4, 11, 12, 13 and 14), day 3 (cat 7), day 4 (cat 6) and day 6 (cat 5), see Table 6. No correlation was found between vaccination date and development of one of the fURI signs using a Spearman's rho test ($p > 0.05$).

	Vaccination day	Start fURI signs
Diet A		
Cat 3	Day 2	-
Cat 5	Day 6	Day 3
Cat 11	Day 2	Day 2
Cat 13	Day 2	Day 3
Diet B		
Cat 4	Day 2	Day 2
Cat 6	Day 4	Day 1
Cat 7	Day 3	Day 7
Cat 12	Day 2	Day 5
Cat 14	Day 2	Day 3

Table 6 Vaccination and start of one or more fURI signs for all cats of the control group (N=4) and the experimental group (N=5) during the 14 day observation period.

A linear mixed model showed no correlation between surrender type, gender, age, weight loss and food intake and fURI scores for ocular discharge, sneezing, nasal discharge and respiration. Wilcoxon signed rank test showed a significant difference in overall ocular discharge scores between day 1 and day 14 (z= -2.449, $p = 0.014$), but not for sneezing scores. On day 12 a significant difference was found in sneezing scores between the quarantine rooms using a Kruskal-Wallis H-test ($\chi^2(2)= 6.6$, $p = 0.037$). Significantly more

scores for sneezing were given to cats in quarantine room B that day. In quarantine room G none of the cats (cat 4 and cat 7) did not show any sneezing signs during the entire 14 day observation period (Fig. 16).

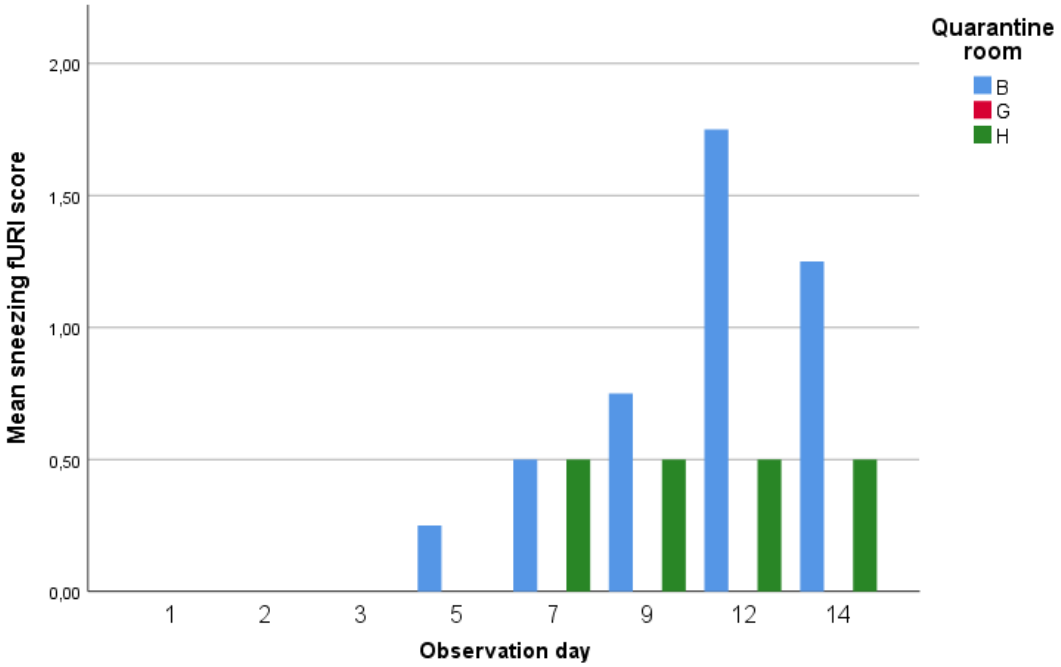

Fig. 16 Mean fURI scores for the clinical sign "sneezing" in quarantine room B (N=4), G (N=3) and H (N=2) on observation days 1, 2, 3, 5, 7, 9, 12 and 14.

Body weight

Body weights were registered for all cats in the control group (4) and for all cats in the experimental group (5). Body weights were noted on day 1 (week 0), day 7 (week 1) and day 14 (week 2). A Shapiro-Wilk test showed a normal distribution for the control group (D(4) = 0.382, p = 0.057) and a non-normal distribution for the experimental group (D(5) = 0.407, p = 0.018). Using boxplots, outliers were found in the experimental group: cat 7 on day 1, 7 and 14 with a mean body weight of 6,42kg and cat 14 on day 7 and 14 with a mean body weight of 2,30kg. No outliers were found in the control group.
Median body weights were determined for both study groups (Fig. 17). Median overall body weight was 2.98kg (IQR = 0.29) for the control group and 3.00kg (IQR = 0.38) for the experimental group. Mann Whitney-U tests revealed no significant differences between both groups for mean weight on day 1 (U = 10.00, z = 0, p = 1,00), day 7 (U = 9.00, z = -0.247, p = 0.841), day 14 (U = 7.00, z = -0.735, p = 0.556) and overall body weights (U = 7.00, z = -0.738, p = 0.508). All cats had a body condition score (BCS) of 4/9 or 5/9 except for cat 3 and 13 which were scored a BCS of 3/9.

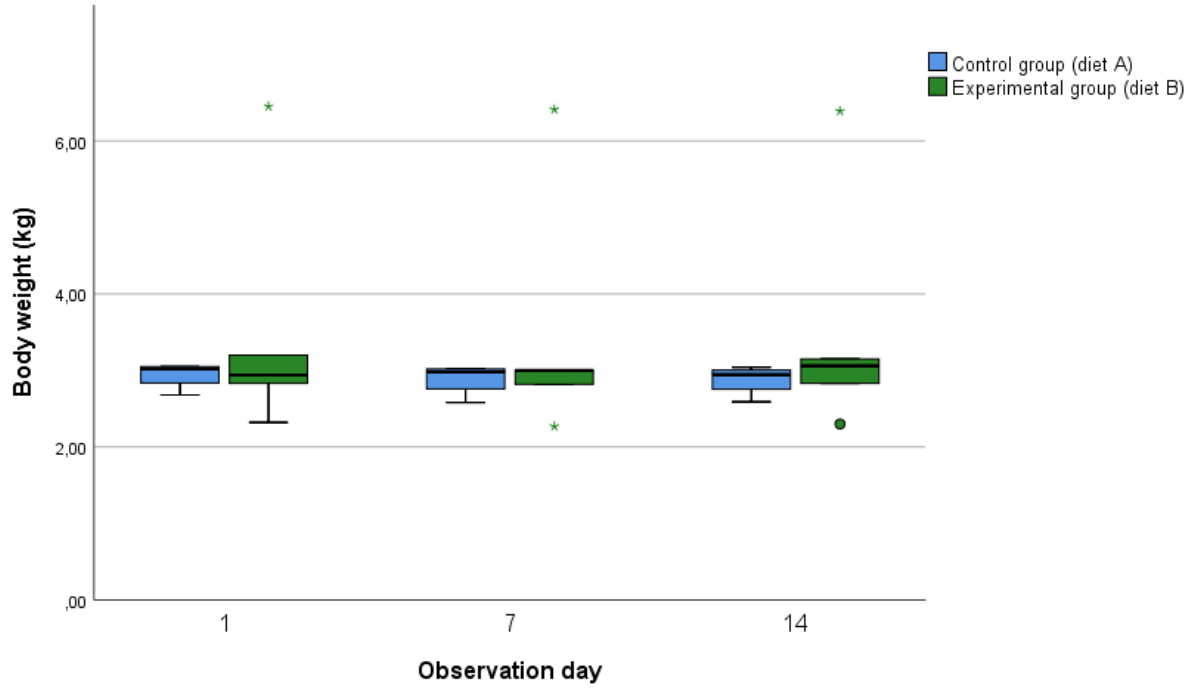

Fig. 17 Mean body weights in kg (± SEM) of the control group (N=4) and the experimental group (N=5) on observation days 1, 7 and 14.

A linear mixed model was executed to detect any significant correlations between bodyweight, time, gender, age, surrender type and treatment group. Treatment group, time, gender and surrender type were set as fixed factors, age as covariate and cat as subject. A significant positive correlation was found for gender (p = 0.00), but not for any other variable. Wilcoxon signed rank tests did not show significant differences between body weight on day 1 and day 7 (z = -1.660, p > 0.05), between day 1 and day 14 (z = -1.402, p > 0.05) and between day 7 and day 14 (z = -0.831, p > 0.05).

Body weight loss/gain

Body weight loss was calculated as a percentage of the initial body weight at day 1 (Fig. 18). Loss or gain of body weight was registered on day 7 (week 1) and 14 (week 2) for all 9 cats. A Shapiro-Wilk test showed normal distributions for weight loss on day 7 and day 14 for both groups (control group: D(4) = 0.0.897, p = 0.415; experimental group: D(5) = 0.868, p = 0.257). Cat 6 and cat 12 were found to be outliers on day 14. The homogeneity of variances was tested using a Levene's test, with a p value of 0.05. For the control and the experimental group the variances were found to be equal (F(1,9) < 0.05, p > 0.05).

In the first week cats in the control group lost an average of 2% (SD = 2.3) and cats in the control group lost an average of 1% (SD = 3.1). An independent-means t-test showed that this was not a significant difference (t(9) = -0.20, p > 0.05. In the second week average weight loss in the control group was 2% (SD = 2.1). In the experimental group there was a small weight gain of 0.19% (SD = 4.2) in the second week. Differences in weight loss and gain between both groups in the second week were also found to be not significant (t(9) = -0.97, p > 0.05).

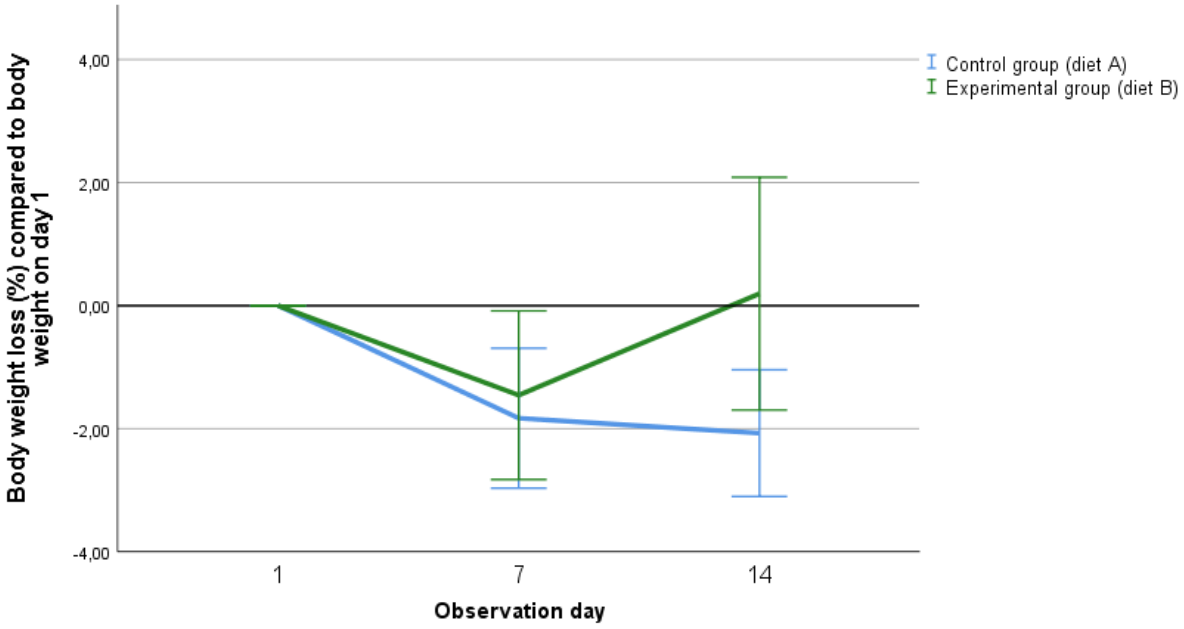

Fig. 18 Body weight loss (± SEM) in % of the initial body weight on day 1 for the control group (N=4) and the experimental group (N=5) on observation days 1, 7 and 14.

Weight losses in the control group ranged from 1% to 4% in the first week and from 0% to 4% in the second week. Cat 5 gained some weight in the first week (1%), but lost weight (0.7%) in the second week. Within-group variations in the experimental group were larger. In the experimental group weight losses varied from 0% to 6% and from 0% to 4% on day 7 and day 14 respectively. Cat 6 gained weight, 2% in the first week and 7% in the second week (Fig. 19).
Overall 6 out of 9 cats (67%) lost weight during the 14 day quarantine period compared with day 1, 2 out of 9 cats (22%) did not gain or lose any weight (compared with day 1) and one cat (11%) gained weight (Fig. 20 and Fig. 21).

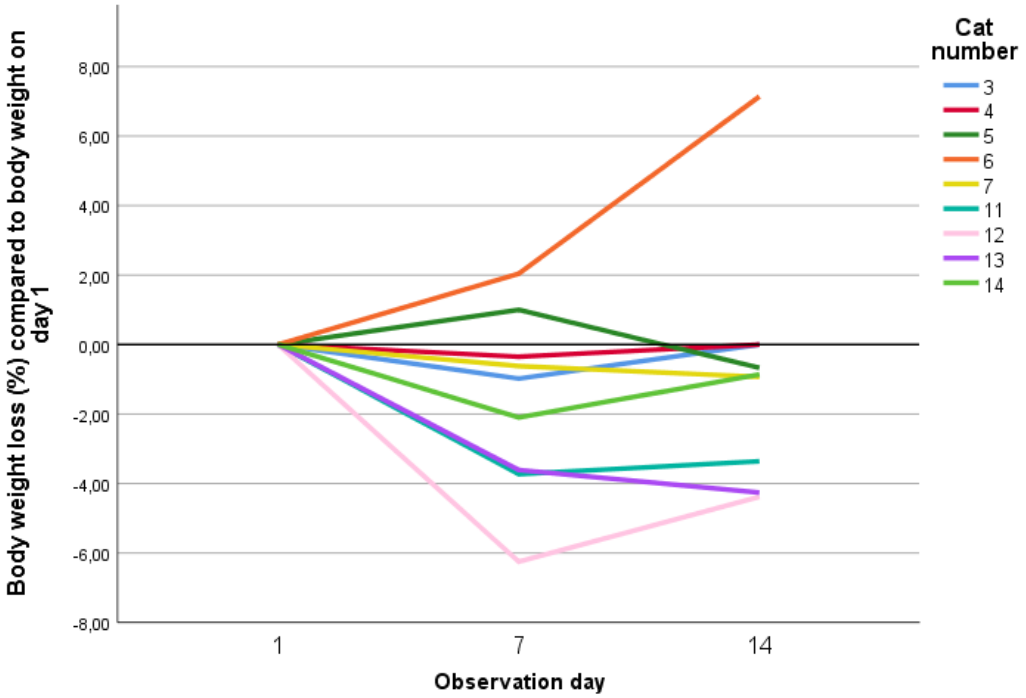

Fig. 19 Body weight loss in % of the initial body weight on day 1 for all cats (N=9) on observation days 1, 7 and 14.

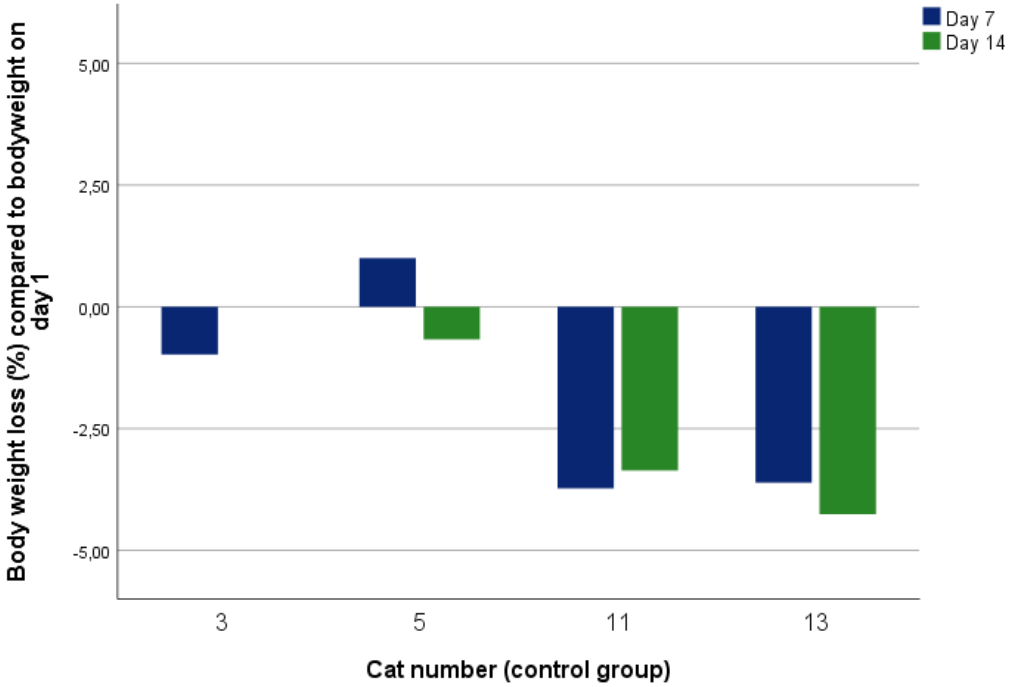

Fig. 20 Body weight loss in % of the initial body weight on day 1 for each cat of the control group (N=4) on observation days 7 and 14.

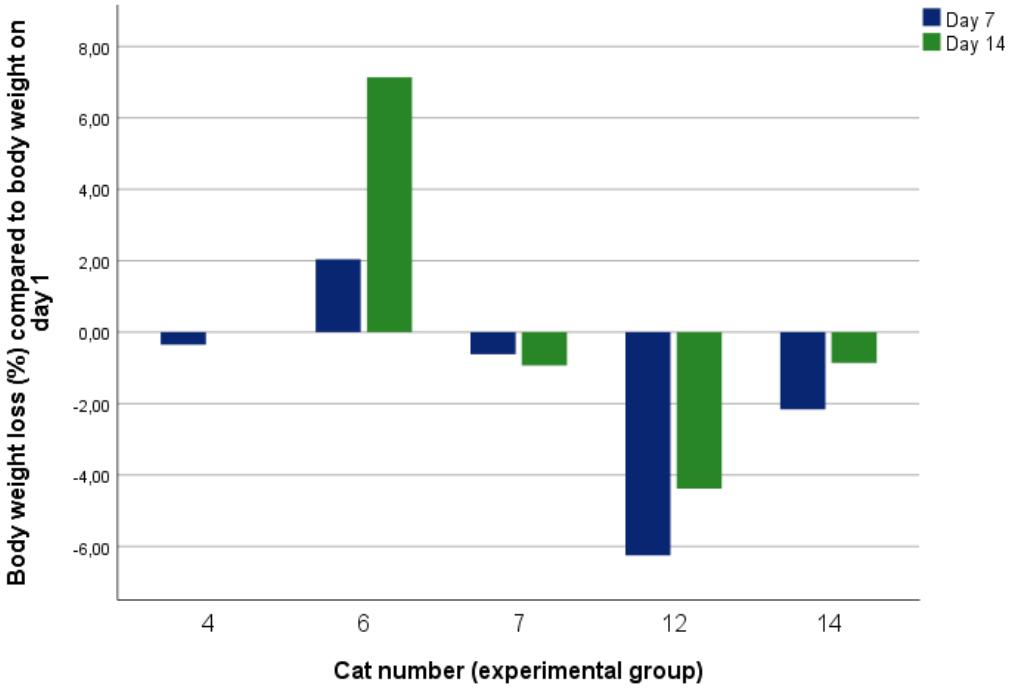

Fig. 21 Body weight loss in % of the initial body weight on day 1 for each cat of the experimental group (N=5) on observation days 7 and 14.

Weight loss during the entire 14 day observation period was ranked according to Tanaka et al. (2012)[7]. None of the cats from this study lost 5-10% or more than 10% of their bodyweight. 6 out of 9 cats lost 0.1-4.9% of their bodyweight and 2 out of 9 cats did not lose any weight.
Body condition scores did not differ during the 14 day observation period for all cats.

Food – and water intake

Daily food intake was registered as a percentage of the daily offered amount of food. A total mean food intake was calculated per research group per day for the 14 day observation period (Fig. 22), as well as a mean food intake per cat. “Morning intake” shows the food intake during the night, whereas “afternoon intake” shows the food intake during the day. All cats were fed more (116%) than their daily requirement, the amount needed to maintain weight (86% of the offered ration) was calculated as a percentage of the daily offered amount of food. Food intake for the control and the experimental group were found to be non-normally distributed using a Shapiro-Wilk test (control group: $D(56) = 0.831$, $p < 0.05$, experimental group: $D(70) = 0.917$, $p < 0.05$). Using boxplots, outliers were found on day 1 (cat 6 and cat 7, experimental group) and on day 6 (cat 6, experimental group), see Fig. 22.

Cat 3, 11 and 13 from the control group and cat 4, 12 and 14 from the experimental group were castrated on day 6 and were not offered any food that morning, so food intake in the afternoon was 0%. Food intake of the control group was at its lowest on day 1 with a median of 29% (± IQR = 67.9) and on day 7 for the experimental group with a median of 35% (± IQR = 65.3). Median food intake during the total 14 day observation period in the control group was 85% (± IQR = 29.8). Median food intake for the experimental group was 73% (± IQR =

50.0). A Mann-Whitney U test showed no significant difference between cats fed diet A (control group) and cats fed diet B (experimental group) (U = 1630, z = -1,634, *p* = 0.102).

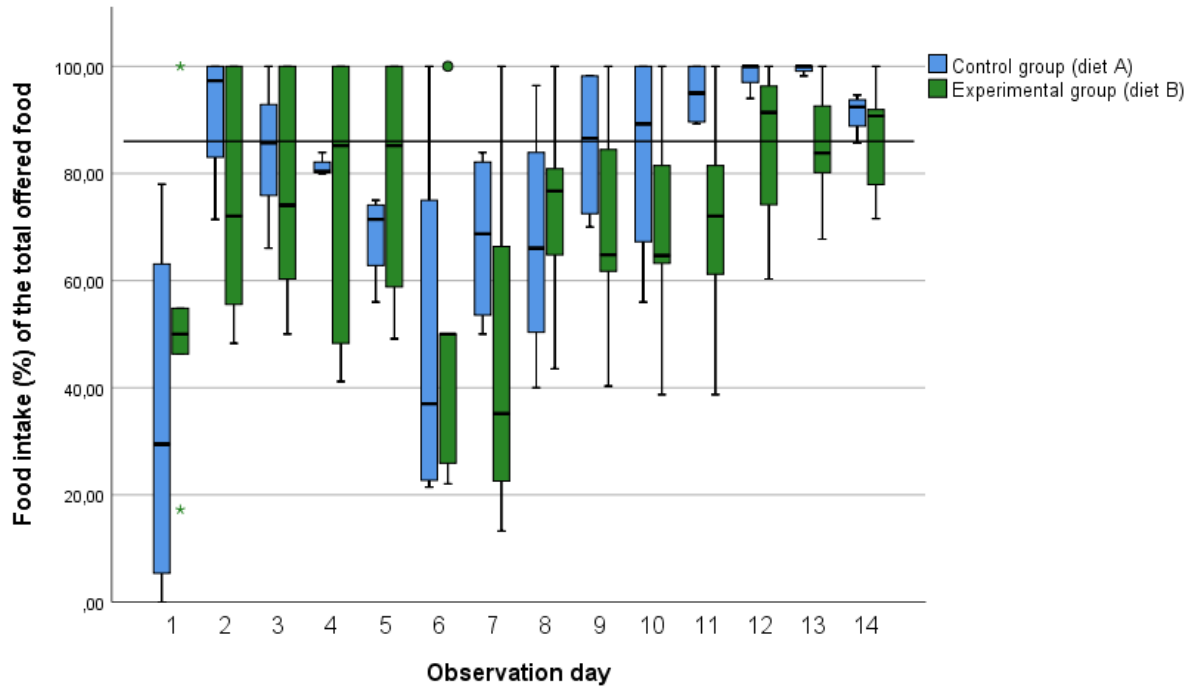

Fig. 22 Clustered boxplot with mean daily food intake in % of the daily offered food (± IQR) for the control group (N=4) and the experimental group (N=5) for observation days 1 to 14, with the daily required food intake set at 86% (black line).

A linear mixed model found no significant correlations for food and water intake with treatment group, gender, surrender type and weight as fixed factors, age as covariate and cat as subject. A negative correlation was found between food intake and time spent in the hiding box (place preference) using a paired samples correlations test (t(9) = -0.677, *p* = 0.045).
Subsequently food intake was calculated for morning and afternoon meals, see Fig. 23 and Fig. 24. Median food intake during the 14 day observation period in the morning was 100% (± IQR = 0.8) for the control group and 100% (± IQR = 8.8). Median food intake in the afternoon was 75% (± IQR = 54.5) and 55% (± IQR = 74.6) for control and experimental group respectively. No significant difference was found between both research groups for morning and afternoon food intake using a Mann-Whitney U-test (morning: U = 1836, z = - 0.723, *p* = 0.470, afternoon: U = 1680, z = - 1,386, *p* = 0.166). A Wilcoxon signed Rank-test showed a significant difference in food intake between morning and afternoon for the control group (z = -4.921, $p < 0.05$) and the experimental group (z = -5.587, $p < 0.05$).

No outliers were found for the control group using boxplots, seen Fig. 23. In the experimental group multiple outliers were found using boxplots. Cat 12 was found to be an outlier on day 3, 4, 5, 12 and 14. Cat 6 was an outlier on day 6, 7 and 8. Cat 4 was an outlier on day 8, 9, 10, 11 and 13, see Fig. 24.

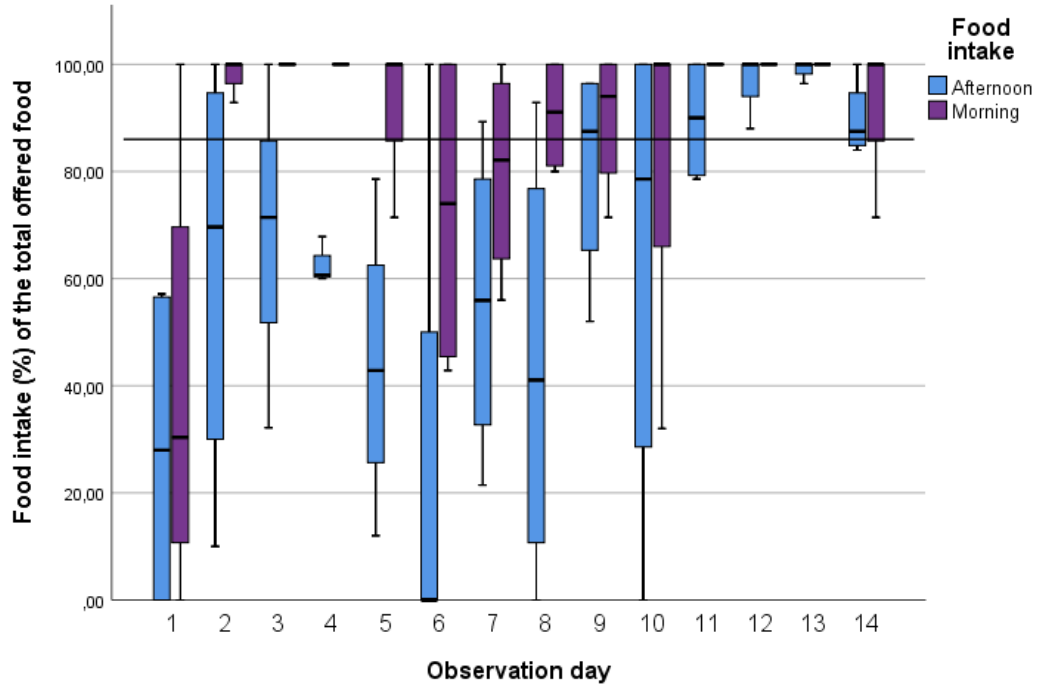

Fig. 23 Clustered boxplot with daily morning and afternoon food intake in % of daily offered food (± IQR) for the control group (N=4), daily required food intake set at 86% (black line).

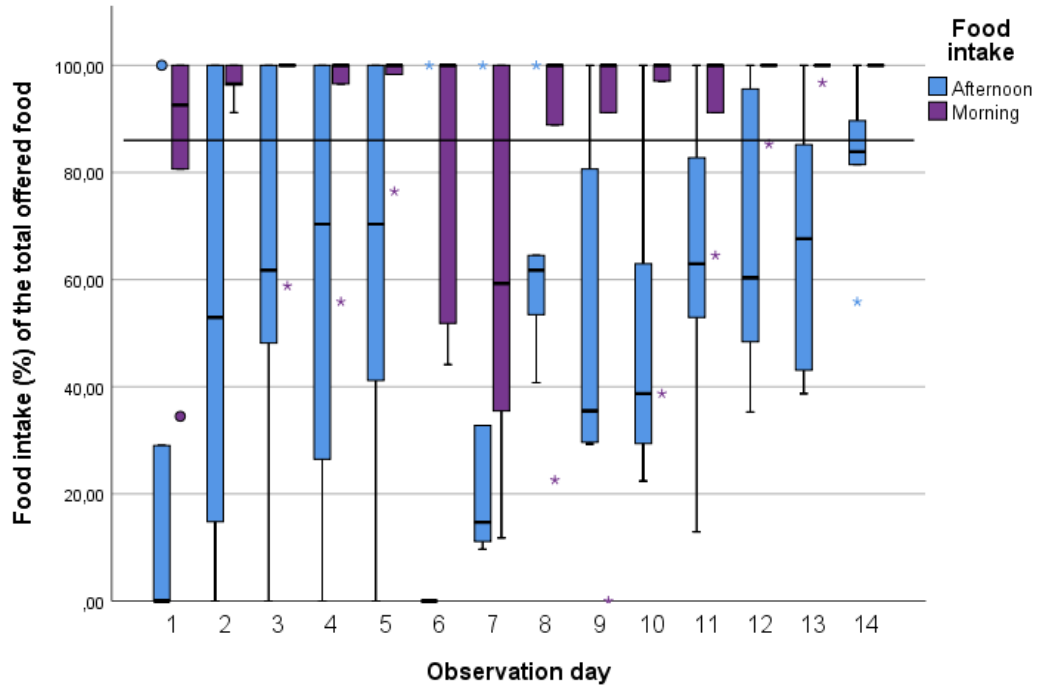

Fig. 24 Clustered boxplot with daily morning and afternoon food intake in % of daily offered food (± IQR) for the experimental group (N-5), daily required food intake set at 86% (black line).

Water intake was registered as a percentage of the total offered amount of water, which was either 200ml or 250ml. Daily water requirement for cats is 44-66ml per kg body weight per day[47]. Both diets offer 5,5% of moisture (Table 2), which was corrected for by using a daily water requirement of 43-65ml per kg body weight per day. As seen in Fig. 25 both research groups remain under the lower limit of the daily water requirement for most of the

time. Mean water intake was significantly less than the lower limit for both groups using an independent-means t-test (t(9) = -4.75, *p* = 0.001).

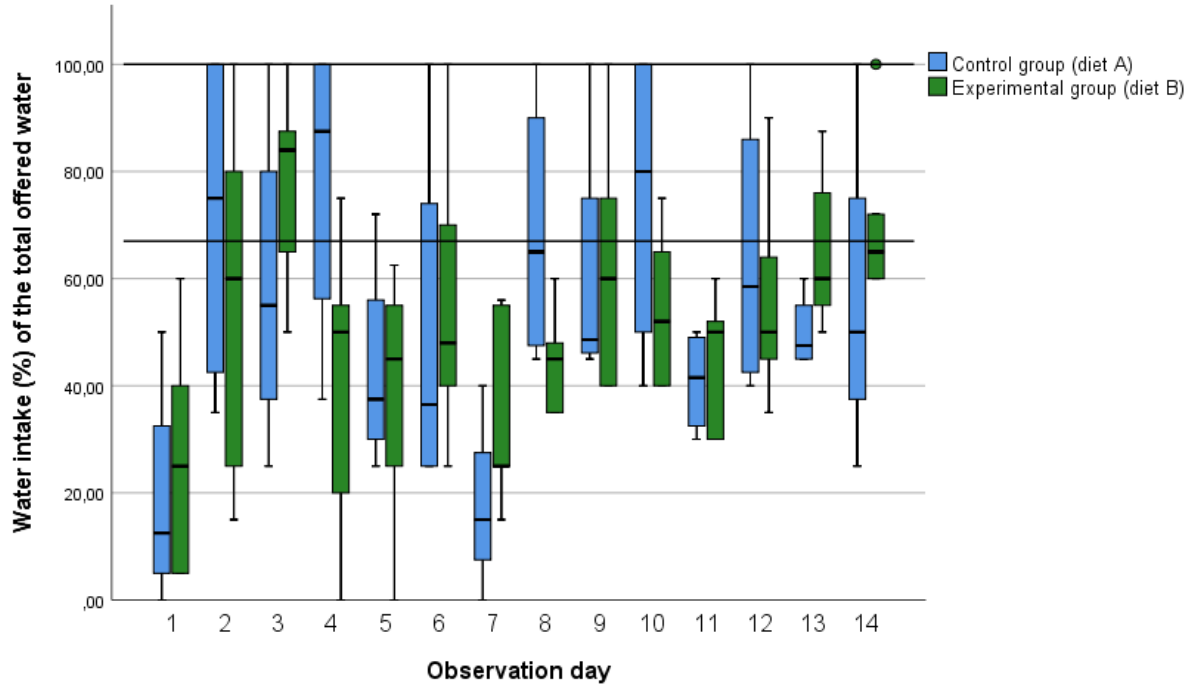

Fig. 25 Mean daily water intake in % of the daily offered water for the control group (N=4) and the experimental group (N=5) for observation days 1 to 14. The lower black line represents the lower limit for daily water requirement (67%), the upper black represents the upper limit for daily water requirement (100%).

Some cats threw water bowls over or threw one of the towels in the water bowl. Which happened on day 2, 3, 4, 5, 6, 8, 9, 10, 12 and 14 in the control group and on day 2, 3, 6 and 14 in the experimental group. An independent samples-test showed a significant difference (t(56,70) = 2.93, *p* = 0.004) between the control and the experimental group in the amount of times of throwing over the water bowl. Cats in the control group threw their water bowls over more often than cats in the experimental group, which means mean water intake of the control group would be lower when this would not have happened.

Shapiro-Wilk test showed normal distributions for water intake for the control (D(56) = 0.967, *p* = 0.820) and the experimental group (D(70) = 0.806, *p* = 0.091). Equality of variances was validated by a Levene's test (F(1,9) = 2.512, *p* = 0.157). Water intake was at its lowest for the experimental group on day 1 (27%, SD = 23.6) and on day 7 for the control group (18%, SD = 16.6). Overall, mean water intake for the control and the experimental group was 54% (SD = 12.8) and 52% (SD = 5.9) respectively. No significant differences were found using an independent-samples t-test (t(9) = 0.254, *p* > 0.05).
A food : water ratio was calculated. In a normal situation this ratio should be 1 : 2.7 to 1 : 3.5 (using 44ml to 66 ml water per kg body weight per day). In Fig. 26 this ratio is shown for diet A (control group) and diet B (experimental group). A non-normal distribution was found for both groups using a Shapiro-Wilk test (control group (D56) = 0.716, *p* > 0.05, experimental group (D70) = 0.650, *p* < 0.05). The median food : water ratio of the control group was 1 : 2.18 (± IQR = 2.1) and for the experimental group 1 : 2.04 (± IQR = 1.3). A Mann-Witney U-test was used to test for significant differences. No significant difference was found

between both research groups (U = 1750, z = -1.03, p = 0.304). A related samples Wilcoxon signed-rank test showed significant differences between the control group and the upper limit ratio (z = -3.15, p < 0.05) and between the experimental group and both the upper and lower limit ratio (lower limit: z = -3.51, p < 0.05, upper limit: z = -5.73, p < 0.05). Between the control group and the lower limit ratio the Wilcoxon signed-rank test showed no significant difference (z = -0.33, p = 0.738).

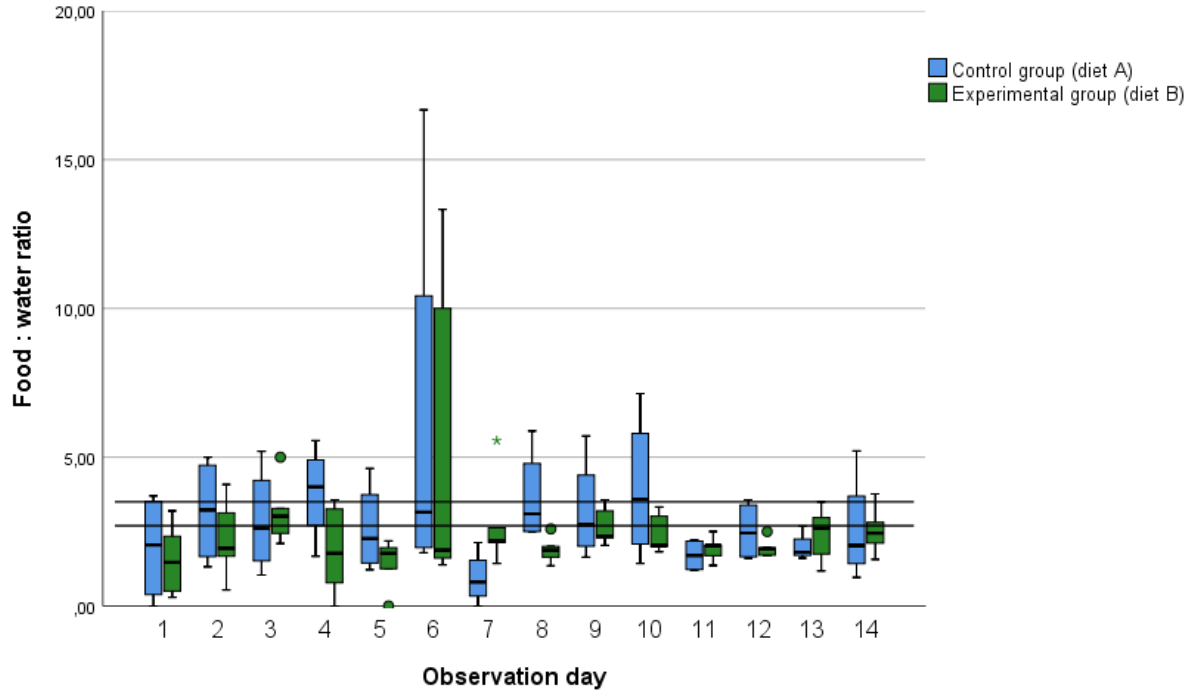

Fig. 26 Clustered boxplot with Food : water Ratio (±IQR) for the control group (N=4) and the experimental group (N=5) for observation days 1 to 14. Lower limit ratio set at 1 : 2.70 (lower black line) and upper limit ratio at 1 : 3.50 (upper black line).

Length of Stay

All cats (100%) were adopted. Cat 5 and 6 had to stay in quarantine for a longer period of time because of a cat with dermatophytosis in their quarantine room. Length of stay was defined for those cats starting from the date they were put up for possible adoption (after 21 days). Mean length of stay was 15 days (SD = 16.1) for the control group and 20 days (SD = 18.6) for the experimental group. A Shapiro-Wilk test showed data from both groups were normally distributed (control group: D(4) = 0.904, p = 0.449, experimental group: D(5) = 0.910, p = 0.446). Equality of variances was validated with a Levene's test (F(1,9) = 0.175, p = 0.688). No significant differences were found using an independent-means t-test (t(9) = -0.480, p = 0.646). The control group had a median of 13 days with a range of 0 to 34 days. The experimental group had a median of 23 days with a range of 0 to 42 days (Fig. 27). No outliers were found in both groups.

A linear mixed model did not show any correlation between length of stay and gender, age, surrender type or treatment group. Cat 11, 12 and 14 were longhair cats (no distinct breed), whereas all other cats were regular European shorthair cats. A significant correlation was

found between hair length and adoption rates using a linear mixed model (t(9) = 3.086, *p* = 0.018).

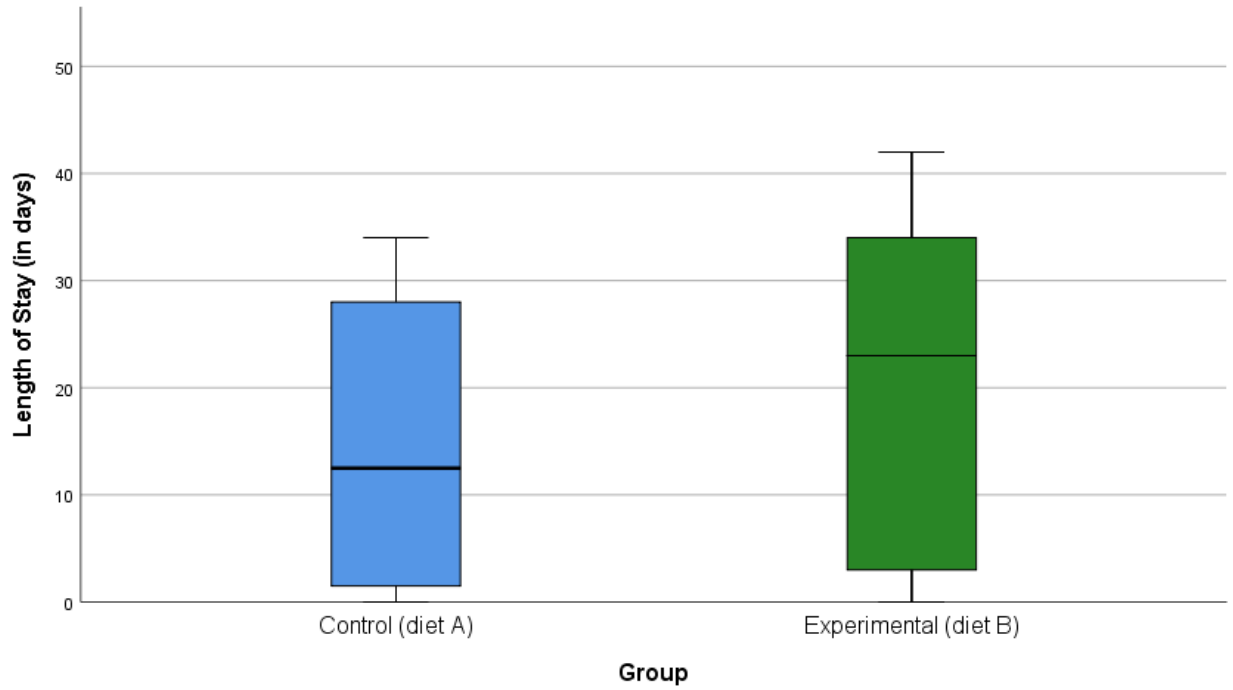

Fig. 27 Median (± IQR) adoption rates in days of the control group (N=4) and the experimental group (N=5).

Discussion

The aim of the study was to evaluate the effect of a diet supplemented with alpha-casozepine and L-tryptophan (Royal Canin Veterinary Diet® Calm ™ cat) on place preference, body weight, and development of fURI signs in newly arrived cats during the 14 day quarantine period in a Dutch animal shelter. The adoption dates were registered to give insight in possible differences in the Length of Stay.
Considering previous research, it was hypothesized that the supplemented diet would significantly reduce stress levels in newly arrived cats compared to the control group, resulting in a faster adaptation to the novel shelter environment. As high stress levels for a longer period of time in cats can result in weight loss, cats who were fed the supplemented diet could lose less weight compared to the control group. Since prolonged stress affects the immune system in a negative way, we expected fURI scores to be significantly higher in the control group. Additionally, we expected cats who were fed the supplemented diet to be more relaxed among humans, making them more eligible, which would result in shorter Length of Stay.

Most important findings of this study were:

- Inter-animal variations were high in place preference, with some of the cats spending almost the entire 160 minutes observation time in one place (e.g. in their hiding box).
- Cats in both groups spend most of their time in their hiding box, but no significant difference in place preference was seen between both research groups. Place preference on day 1 seemed to be random.
- Sneezing and ocular discharge were most often seen as fURI sign and scores did not differ between both research groups, but did significantly correlate with quarantine room.
- Weight loss was less increased compared to previous studies and was not significant between day 1 and day 14 in both the control and the experimental group.
- A negative correlation was found between time spend in the hiding box (of the total observed 160 minutes) and food intake.
- Food and water intake in all cats were significantly lower than the daily requirements. No significant differences were found for food and water intake between both groups.

Place preference

Cats prefer elevated area's and hiding spaces to adapt when exposed to stressful situations[8,16]. This explains both research groups spending most of their time in their hiding box during the 14 day observation period (control group: 39.99%; experimental group: 72.46%). Corresponding with the studies by Selman et al. (2017) and Vinke et al. (2014) where cats who were offered a hiding box, spend most of their time in that hiding box. Previous studies showed a decline in time spent in the hiding box on day 2 compared to day 1[20,49]. Although stress scores might be at its highest on the first day according to these studies, we did not find a decrease in the use of the hiding box in both groups on the first days. Cats in both groups used their hiding box more on day 2 than on day 1. This was corresponding with the study of Rehnberg et al. (2015) where cats choose their favoured areas more randomly during the first day.

this assessment was performed by the cats owners who might not be familiar with all visual signs of stress. Additionally cats stayed in their caregivers homes instead of quarantine cages, which might be less stressful.

In the study by Beata et al. (2007) no significant differences were found between the control group and the experimental group (supplemented with alpha-casozepine) in fearful behaviour, autonomic signs and in owner evaluations regarding stress-related behaviour. Cats in the present study were housed in a shelter environment instead of in the home of a caregiver, thereby encountering more stressors. We expected cats to use their hiding box less when fed the experimental diet, as it was hypothesized that the supplemented diet would reduce stress levels. No significant difference was found between the control and the experimental group for the time spend in the hiding box. This would indicate that the supplemented diet did not reduce stress levels enough to see any behavioural changes, which would correspond with the study by Beata et al. (2007).

As seen in previous studies, cats that did not receive a hiding box showed alternative hiding behaviour by hiding in or behind their litterbox[6,20,49]. In this study all cats received a hiding box, but alternative hiding (behind the litterbox) was seen in two cats from the control group. Alternative hiding despite being offered a hiding box was also seen by Selman et al. (2017). When hiding behind the litterbox was combined with hiding in the hiding box in this study, the amount of hiding behaviour in the control group would be comparable with the experimental group.
On day 7 a significant difference between the control group and the experimental group was found for time spent in the hiding box and time spent on the perching shelf. This could be explained by the castrations of the relinquished cats in both groups that took place on day 6. Besides the fact that castration is a stressful event which could interfere with place preference, when put back in their cages on day 6 in the afternoon they might have had another period of randomly choosing their favoured area as seen on day 1.

Perching shelfs add vertical space an provide cats with a place to observe their surroundings and may help in reducing anxiety and stress[8,52]. In this study the perching shelf was used for 32% of the time in the control group and for 18% of the time in the experimental group and was most preferred after “hiding box”. Variations between cats (17% to 99% for the hiding box, 0% to 62% for the perching shelf and 0% to 43% for being elsewhere) were high and indicated individual preferences[8,20]. These results are supported by Ellis et al. (2017) and the influence of these within-group differences could have had a large influence on the results because of the small sample size (N=9)[53,54]. As for the observation time (20minutes), this could have caused dissimilarities as some cats were sleeping in one place for the complete 20 minutes observation time. This could be reduced by observing 2 times a day as in the study by Suchak et al. (2016)[55].

Feline upper respiratory infection (fURI) scores
According to the definition of upper respiratory tract infections by Dinnage et al. (2009) all but one cat developed fURI during the 14 day observation period. This could have been a slight exaggeration as serous eye and nose discharge is not specific for fURI, but was defined as an upper respiratory tract infections by using the definition of Dinnage et al. (2009). The scoring method for fURI scores was quite objective. Sneezing was sometimes difficult to see

when cats were in their hiding box or behind their litterbox. Cat 7 was anxious for human contact and turned his head the other way whilst in his hiding box, making it even more difficult to score possible fURI signs.
No significant differences in fURI signs were found between the control and the experimental group, indicating that the supplemented diet did not prevent or lower the development of upper respiratory tract infections. A significant difference was found between day 1 and day 14 of the ocular discharge scores, indicating that indeed some kind of upper respiratory infection has developed. Cats were vaccinated (Felocell® CVR) on day 2 (cat 3, 4, 11, 12, 13 and 14), day 3 (cat 7), day 4 (cat 6) and day 6 (cat 5). No correlation was found between vaccination date and the development of one or more fURI signs. Indicating vaccination did not have any influence on the development of feline upper respiratory infections in this study.
In quarantine room B significantly more cats were seen sneezing, without a difference between the control or the experimental group. This could be because of high viral shedding in a contained area. Quarantine room B was the most occupied quarantine room (N = 4), whereas quarantine room G housed 3 cats and quarantine room H only 2 cats, which could play a role in viral load.

Dybdall et al. (2007) found relinquished cats showing more behavioural stress than strays, as they go through a more recent stress. And even though strays might suffer from incomplete nutrition and are more likely to have an incomplete vaccination status, they adapted quicker to changes in their environment and were able to fight the infection better[56]. Dinnage et al. (2009), however, found stray cats having a higher risk on developing fURI, which would mean nutrition and a possible infection before entering the shelter might play a role in the development of fURI in an animal shelter. A correlation between fURI scores and age was also seen in the study by Dinnage et al. (2009), older cats were at higher risk of developing fURI. In this study we found no correlation between fURI scores and surrender type nor did we find a correlation with age, gender, weight loss or food intake.

Incubation time for FCV and FHV reflects the time to develop fURI and is 2-10 days and 2-6 days respectively. Time for recrudescence, which is the development of disease after reactivation, is 4-11 days for FHV[25,57,58]. As all cats in this study were either not optimally vaccinated or had an unknown vaccination status it was not clear if an optimal vaccination status would prevent the development of fURI. 8 out of 9 cats developed fURI signs at day 7 in this study, which is in line with the time to develop fURI signs found as aforementioned. No correlation was found between vaccination date and first clinical signs of fURI. This, and data from previous studies, shows stress is a risk factor for developing fURI signs when entering a shelter. Possibly because of reactivation of previous infections and new infections as a result of suboptimal housing (over-crowding, hygiene) in shelters[23]. Especially reactivation of latent infections has been found as a major factor in upper respiratory tract infections in animal shelters as 80-100% of cats following initial infection becomes latently infected and 45% of these cats will re-shed after a stressful event[59]. Bannasch et al (2004) found a URI rate peak around day 13-20 in a shelter, after which the rates declined. It would be interesting to see what would happen with fURI rates during longer stays when cats are provided with a hiding box. And it would be interesting to see if the transfer from quarantine room to adoption area would have an influence. Quarantine would allow newly introduced animals developing fURI signs during quarantine before they would be exposed to healthy

cats at the adoption floor. The shelter in this study kept cats without a clear vaccination history and stray cats in quarantine until they had received both vaccinations, which is longer than the obligatory two weeks. This is an efficient method, though not all shelters will have the capacity to hold cats in quarantine for more than three weeks.

Body weight

Body weights did not differ significantly within-groups, apart from cat 7, which was the only tomcat in this study and weighed (6.45kg). This was almost twice as much as the weight of all other (female) cats upon arrival.

Stress can cause a loss of appetite resulting in loss of body weight[14,16]. During the 14 days in quarantine, loss of body weight was reported for 67% of all cats (6 out of 9), which is lower than in the study by Selman et al. (2017) and the study by Bidlot et al (2018)[20]. Loss of body weight was not prevented by the supplemented diet. The supplemented diet was not able to ensure cats to maintain their bodyweight, which indicates it has no effect on stress levels in cats in an shelter environment. A longer study period, as used in the study Miyaji et al. (2015), could be used in further studies to find possible significant effects of a supplemented diet. For shelter cats this could be an issue, as reduction of stress should take place as quick as possible because of the often relatively short stay in the shelter.

Body weight loss on day 14 compared to day 1 was 2% in the control group and even though there was a weight gain of 0.19% in the experimental group the difference between both groups was not significant. This limited body weight loss was in contrast to the previous study by Selman et al. (2017) where a mean body weight loss of 5% was found. Bidlot et al. (2018) found a body weight loss of 2%, conform the loss found in this study. These studies used a hiding box in their experimental group and provided them a non-supplemented diet, which resembles the control group in this study.
Loss of body weight of all cats in this study ranged from 0% to 4%. Cat 6 (experimental group) gained weight in both weeks (2% in the first week compared to day 1 and 7% in the second week compared to day 1), though the body condition score of this cat did not indicate being underweight (BCS 4/9) upon arrival. Cat 3 (control group) and cat 4 (experimental group) lost less than 1% of their body weight in the first week compared to day 1, but gained this 1% loss of body weight in the second week (no gain or loss of body weight on day 14 compared to day 1). Cat 12 (experimental group) lost the largest amount of weight (6% in the first week compared to day 1 and 4% in the second week compared to day 1) during the 14 day observation period.

Food and water intake

Decreased food and water intake or anorexia can be seen in cats exposed to stress. As well as inhibition of elimination of faeces and urine[7,19]. Mean food intake on day 1 in this study was 45% (SD = 32.05), cat 5 not eating any food at all on that day. In contrast with Tanaka et al. (2012) where 34% did not eat on day 1 and 84% of those cats did not eat on day 2, all cats (100%) in this study ate on day 2. In the study of Tanaka et al. (2012) no hiding places or perching shelfs were offered which could explain this discrepancy. When compared to the study by Bidlot et al. (2018) which did use these enrichments (hiding box, perching shelf), food intake is similar.

After day 1 food intake increased in both groups, though no significant difference was seen between the experimental group and the control group as in the study by Bidlot et al. (2018). The supplemented diet did not provide a faster recovery in food intake nor did it show any improvement in food intake in relation to the control group.

Lowest food intake for the experimental group was on day 7 (47%, SD = 35.56), whilst in previous studies lowest food intake for each research group was on day 1 and 2. This could be explained because of the castration on day 6 in this study for 3 out of 5 cats in the experimental group. Cat 4 (experimental group) ate 100% for day 2 to day 5, but recovered slowly from her castration and needed NSAID's for 4 subsequent days. Food intake reached > 60% on day 12 for this cat.
Food intake in both groups was significantly higher in the morning than in the afternoon. The food which was measured in the morning was placed in the cage in the afternoon the day before, allowing the cats to eat during the afternoon and night. Not only was this a longer period of time than during the day (15 hours instead of 9 hours), no staff or visitors were walking across the hallway, offering silence and a restful setting during the night. Cats eating mainly during the afternoon and night corresponds with studies showing activity levels of cats being at its highest around twilight. Stella et al. (2013) found food intake being at its highest around feeding time (in the morning), which would be in contrast with the hypothesis that interference of staff would lower food intake[60]. Though this would be in line with Ellis et al. (2013) who found cats being more active in response to human activity[54].

Overall, cat 12 ate the least of all cats (58%), this was also the cat who spend the most time in the hiding box (99.15%). A negative correlation was found between time spent in the hiding box and food intake. This might be explained by the fact that cats preferred hiding over eating, as eating meant they had to leave their save hiding place and expose themselves. Similar results were found in previous studies[20,49].

Water intake was at its lowest on day 1 for the experimental group and on day 7 for the control group. Though water intake on day 1 and day 7 for the control group was respectively 19% and 18%. On day 2 water intake had already doubled in the experimental group and was almost quadrupled in the control group. Water intake for the experimental group dropped on day 7. This was also seen in food intake and can be explained by the castration on day 6, which also caused a drop in food intake on day 6 (no food was offered that morning, afternoon food intake being 0% for those cats). This shows castration has a major impact in shelter cats on food and water intake.
In contrast with food intake which reached a mean of 89% of the daily offered amount of food on day 14 (86% being the mean daily required amount of food), water intake did not show the same increase. Highest water intake was seen on day 3 in the experimental group (77%), and on day 4 in the control group (78%). Food : water ratio's showed cats in the experimental group having a significantly lower ratio than the lower limit ratio for daily requirements. This means cats in this group did not drink enough water for the amount of food that was eaten. Food : water ratio in the control group did not differ significantly from the lower limit ratio for daily requirements. This means cats in this group drank enough water in relation to food intake, but as food intake is below the daily requirement, water intake is as well. Low water intake can cause health problems, e.g. feline lower urinary tract diseases and is therefore a serious problem[61]. On day 6 food : water ratios are high for both

the control and the experimental group. On this day cat 3, 11 and 13 (control group) and cat 4, 12 and 14 (experimental group) were castrated and were not offered any food in the morning on day 6. Water was always available, even on day 6, which results in a high food : water ratio.

In this study water bowls were often thrown over or towels were thrown in the water bowl. This resulted in excessive water intake results on some days and affected the results as it was not clear how much water was actually taken. For further studies water bowls should be either secured a few centimetres above the floor of the cage to one of the walls of the cage or to the door of the cage to prevent the waste of water.

Length of Stay

Behaviour, such as friendliness towards the adopter and playfulness, of a cat has been seen as one of the most important reasons for adoption. Cat 7 had the longest length of stay with 42 days, which could have been because of its anxious and timid behaviour, but this was also the only male cat. In contrast Sinn et al. (2016) showed that when gender was a criteria in adopting a cat, male cats were preferred. Thus, the anxious behaviour might have been a more important factor in the longer stay of this cat. Cat 11 and 14 were longhair cats and were already reserved before leaving the quarantine room. Most likely because of their appearance and friendly behaviour.

No significant differences in length of stay was found between the experimental and the control group, indicating that the supplemented diet did not causes cats to be more friendly or influence length of stay in any other way. In previous studies appearances affect length of stay barely. When they do have an influence, coat length stands out, in which a short coat length is preferred because of ease of care[41,62]. This is in contrast with the present study. Higher adoption rates were found in cats with long hair. Probably this is because of the fact that long hair cats are not often seen in a shelter and resemble certain popular breeds.

Welfare implications

Stress has a major impact on cat's health. Anorexia or loss of appetite can cause serious weight loss. To ensure feeding each cat enough food an easy formula can be used as used in this study. This formula could be used to make a schedule of the amount of food per kg body weight, making it clear and feasible for employees and volunteers. Water intake should be stimulated as much as possible for example by offering canned food and by making sure water will not be spilled like it does with improperly presented water bowls. Reducing stress is also important to improve welfare of shelter cats. Offering them a hiding box or perching shelf allows them to naturally react on a stressor[6]. Variations between individuals have been observed and this should be considered regarding enrichment and types of housing.

Limitations

The small sample size is one of the limitations in this study, which caused within-group variations to have a large influence on the results. Data collection took place in one animal shelter from July 23 to September 16 (2018). Only newly brought in healthy cats, aged 1-10 years were incorporated in this study, meaning sample size was depending on the number of cats brought in during the data collection period. As cats needed to be observed for two weeks, last intake of new cats was set on September 2 (2018). This resulted in a period of six weeks in which cats could be brought in for this study. This relatively short period could be a reason for the small sample size, along with the data collection taking place in just one shelter. As most cats (stray and relinquished) were brought in from May to September in 2017 in this shelter, the data collection of this study was executed in the most optimal period for gathering as many cats as possible in this shelter, see Appendix 1. For maximizing sample size, future studies could use a longer period for data collection (and therefore a longer period of taking in new cats) and use multiple shelters.

Data collection taking place in one shelter could have resulted in a small sample size, and additionally it is difficult to say if this data can be generalized to other shelters because of this. Animal shelters could differ in their procedures and quarantine set up and therefore give different results. Though similar results to this study's results were found in previous studies executed in different animal shelters (Arnhem and Utrecht, the Netherlands). It would be interesting to compare results of this study to these previous study's to detect any possible significant differences.

Miyaji et al. (2015) found reduced cortisol levels in cats fed the same supplemented diet as used in the present study for 8 weeks. The observation period of this study was 14 days, which could have been too short to detect any significant effects of the supplemented diet. This observation period was set at 14 days, as all cats would have a minimum stay of 14 days in the quarantine area. Novelty stress is at its highest in the first weeks after admission, which makes these weeks the most crucial for reducing stress. Besides, maximum Length of Stay was 42 days in this study. Though it would be interesting to see if the supplemented diet would have any effects in shelter cats when fed for a longer period of time, it is questionable how applicable this would be.

Future work

For further research it would be interesting to see what the effect of the supplemented diet would be on long term (longer than 14 days) and if there would be an effect on stress after transfer to the adoption area. Besides, it would be interesting to see if the supplemented diet would have an effect of adjustment time of cats after being adopted. Cats in this study were either stray or relinquished, none of the cats were fed the supplemented diet upon entering the animal shelter. As being rehomed induces another period of stress, feeding the supplemented diet in the shelter (before being adopted) might help in adjusting quicker.

Conclusion

The present study shows that a diet supplemented with alpha-casozepine and L-tryptophan (Royal Canin Veterinary Diet® Calm™ cat) did not reduce stress levels in shelter cats during the 14 day quarantine period. No significant differences were found between the group fed the supplemented diet and the group fed a regular diet regarding place preference, body weight, the development of fURI signs and LOS. This study shows body weight is a practical and non-invasive way to recognize stress in shelter cats and should be used in further research.

Food and water intake was significantly lower than the required daily amounts for all cats in this study, which implies this is a serious problem for cats in an animal shelter and should be strictly monitored.

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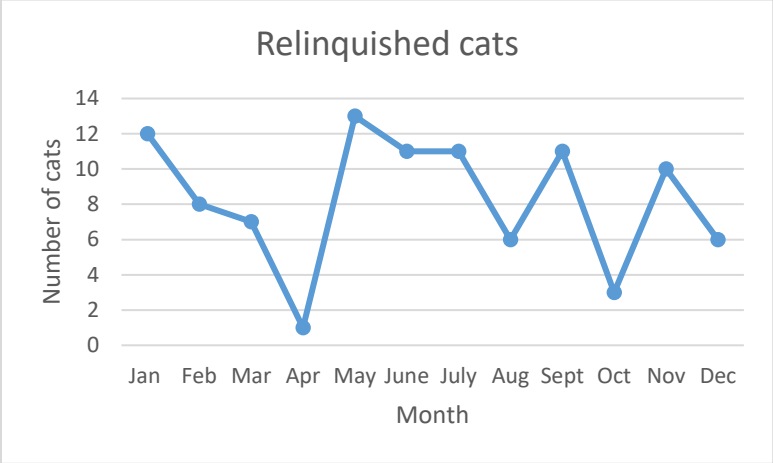
Appendices

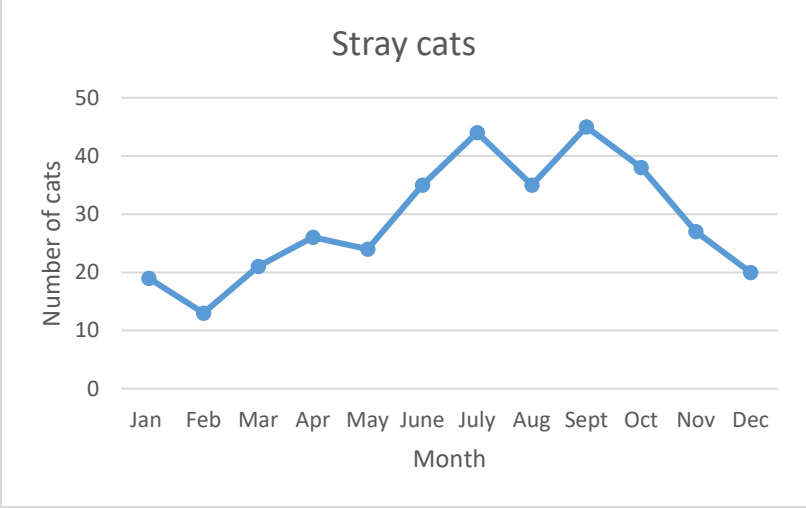
Appendix 1: Data animal shelter “Dierentehuis Stevenshage” 2017

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Relinquishment	12	8	7	1	13	11	11	6	11	3	10	6	99
Stray	19	13	21	26	24	35	44	35	45	38	27	20	347
Returned after placement	4	2	5	5	3	4	1	1	4	4	3	0	36
Exchange	2	2	5	0	0	0	1	0	2	0	0	0	12
Born in shelter	0	0	3	8	0	0	0	15	0	0	0	0	26
Total	37	25	41	40	40	50	57	57	62	45	40	26	520

Amount of cats taken in during 2017 at “Dierentehuis Stevenshage”.



Amount of relinquished cats taken in during 2017 at “Dierentehuis Stevenshage” each month.



Amount of stray cats taken in during 2017 at “Dierentehuis Stevenshage” each month.

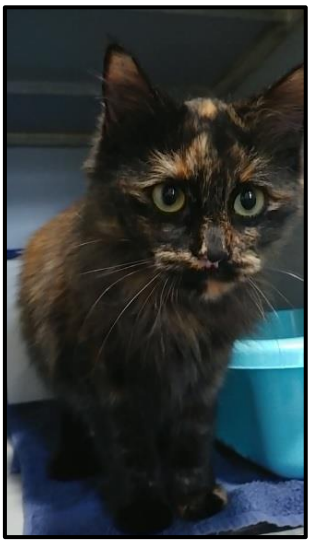
Appendix 2: Participating cats

Control group – Diet A

Cat 3 “Skipper”


Cat 5 “Jannie”


Cat 11 “Janna”


Cat 13 “Stevie”

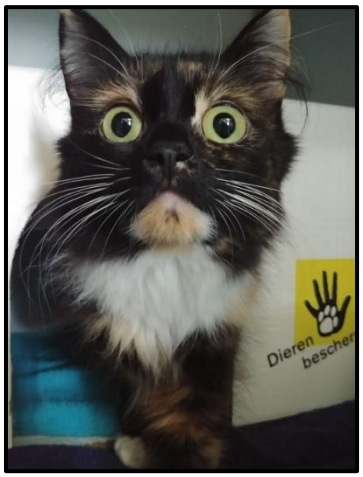
Experimental group – Diet B

Cat 4 “Speedy”

Cat 6 “Sjenkie”

Cat 7 “Grey”

Cat 12 “Tysha”



Cat 14 “Shanai”

Appendix 3: Food and water intake form

Asielnummer		
Datum binnenkomst		
Gewicht		
Dag 1:	Dag 7:	Dag 12:
Hoeveelheid voer per dag (in gram)		
Hoeveelheid voer per keer (in gram)		
Hoeveelheid water (in ml)		

Voeding: overgebleven brokjes afwegen, hoeveelheid noteren en daarna weggooien
Water: water overgieten in een maatbeker, deze hoeveelheid invullen onder kopje “water” en daarna weggooien
Urine: boven de streep ochtend (ja/nee), onder de streep middag (ja/nee)
Ontlasting: boven de streep ochtend (ja/nee), onder de streep middag (ja/nee)
1e ontlasting bewaren in de koelkast (en een vinkje zetten in kolom naast ontlasting)

	Overgebleven voeding (in gram)		Water (in ml)	Urine	Ontlasting		Opmerkingen
Datum	ochtend	middag					
0							
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							

Appendix 4: Place preference per cat per observation day

Diet A

	1	2	3	5	7	9	12	14	**Total (160min)**
Cat 3									
Shelf	100%				48,67%	0,75%	14%	26,92%	23,73%
Hiding box		100%	73,42%	39,33%			3,83%	6,75%	27,92%
Behind Litterbox						18,33%	28,33%		5,83%
In litterbox									0%
Elsewhere			26,58%	60,67%	51,83%	80,92%	53,83%	66,33%	42,52%
Cat 5									
Shelf	21%				5,92%				3,33%
Hiding box	59,33%	60,42%	88,75%	94,00%	37,83%	100%	100%	100%	80,04%
Behind Litterbox									0%
In litterbox									0%
Elsewhere	19,92%	39,58%	11,25%	6%	56,25%				16,63%
Cat 11									
Shelf	100%			100%	100%		18,83%		39,85%
Hiding box		100%	100%				81,17%		35,15%
Behind Litterbox						100%		100%	25%
In litterbox									0%
Elsewhere									0%
Cat 13									
Shelf	96,50%			100%		100%	100%	100%	62,06%
Hiding box		100%	34,75%						16,84%
Behind Litterbox									0%
In litterbox									0%
Elsewhere	3,50%		65,25%		100%				21,09%

Diet B

	1	2	3	5	7	9	12	14	**Total (160min)**
Cat 4									
Shelf								9,33%	1,67%
Hiding box	91,08%	100%	45,50%	78%	85%		100%	72,42%	71,50%
Behind Litterbox									0%
In litterbox									0%
Elsewhere	8,92%		54,50%	22%	15%	100%		18,25%	27,33%
Cat 6									
Shelf	98,25%	23,50%	100%	100%		100%	84,25%		62,25%
Hiding box		47%			100%			45,58%	24,07%
Behind Litterbox									0%
In litterbox									0%
Elsewhere	1,75%	29,50%					15,75%	54,42%	12,68%
Cat 7									
Shelf	100%								12,50%
Hiding box		100%	100%	100%	100%	100%	100%	100%	87,50%
Behind Litterbox									0%
In litterbox									0%
Elsewhere									0%
Cat 12									
Shelf									0%
Hiding box	100%	98,17%	100%	100%	100%	100%	100%	100%	99,77%
Behind Litterbox									0%
In litterbox									0%
Elsewhere		6,83%							0,85%
Cat 14									
Shelf			77,33%	36,92%					10,53%
Hiding box	100%	100%		35,75%	100%	100%	100%	100%	79,47%
Behind Litterbox									0%
In litterbox									0%
Elsewhere			52,67%	27,33%					10%

Appendix 5: feline upper respiratory infection (fURI) scores per cat per day

Control group (Diet A)

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 12	Day 14
Ocular discharge								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	1	1	1	1	1	1
Cat 11	0	1	1	1	1	1	1	1
Cat 13	0	0	1	1	1	1	1	1
Nasal discharge								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	0	0	0	0	0	0
Cat 11	0	0	0	0	0	0	0	0
Cat 13	0	0	0	0	0	0	0	0
Respiration								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	0	0	0	0	0	0
Cat 11	0	0	0	0	0	0	0	0
Cat 13	0	0	0	0	0	0	0	0
Sneezing								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	0	0	1	1	1	1
Cat 11	0	0	0	0	1	1	2	2
Cat 13	0	0	0	1	1	1	2	1
Coughing								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	0	0	0	0	0	0
Cat 11	0	0	0	0	0	0	0	0
Cat 13	0	0	0	0	0	0	0	0
Demeanor								
Cat 3	0	0	0	0	0	0	0	0
Cat 5	0	0	0	0	0	0	0	0
Cat 11	0	0	0	0	0	0	0	0
Cat 13	0	0	0	0	0	0	0	0

Experimental group (Diet B)

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 12	Day 14
Ocular discharge								
Cat 4	0	1	1	1	1	1	1	1
Cat 6	1	1	1	1	1	1	2	1
Cat 7	0	0	0	0	0	0	0	0
Cat 12	0	0	0	1	1	1	1	1
Cat 14	0	0	1	1	1	1	1	1
Nasal discharge								
Cat 4	0	0	0	0	0	0	0	0
Cat 6	0	0	0	0	0	0	0	0
Cat 7	0	0	0	0	1	1	2	1
Cat 12	0	0	0	0	0	0	0	0
Cat 14	0	0	0	0	0	0	0	0
Respiration								
Cat 4	0	0	0	0	0	0	0	0
Cat 6	0	0	0	0	0	0	0	0
Cat 7	0	0	0	0	1	1	0	0
Cat 12	0	0	0	0	0	0	0	0
Cat 14	0	0	0	0	0	0	0	0
Sneezing								
Cat 4	0	0	0	0	0	0	0	0
Cat 6	0	0	0	0	0	0	0	0
Cat 7	0	0	0	0	0	0	0	0
Cat 12	0	0	0	0	0	0	1	0
Cat 14	0	0	0	0	0	1	2	2
Coughing								
Cat 4	0	0	0	0	0	0	0	0
Cat 6	0	0	0	0	0	0	0	0
Cat 7	0	0	0	0	0	0	0	0
Cat 12	0	0	0	0	0	0	0	0
Cat 14	0	0	0	0	0	0	0	0
Demeanor								
Cat 4	0	0	0	0	0	0	0	0
Cat 6	0	0	0	0	0	0	0	0
Cat 7	0	0	0	0	0	0	0	0
Cat 12	0	0	0	0	0	0	0	0
Cat 14	0	0	0	0	0	0	0	0