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The effect of a hiding box on stress levels and body weight in Dutch shelter cats; a randomized controlled trial

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Abstract:	<p>While staying in an animal shelter, cats may suffer from chronic stress which impairs their health and welfare. Offering hiding opportunities can significantly reduce behavioural stress in cats, but confirmation with physical parameters is needed. Therefore, the aim of this study was to determine the effect of a hiding box on behavioural stress levels (scored by means of the Cat-Stress-Score) and a physical parameter as body weight in newly arrived cats in a Dutch animal shelter during the first 12 days in quarantine situations.</p> <p>Twenty three cats between 1 and 10 years of age were randomly divided between the experimental (N = 12) and control group (N = 11) with and without a hiding box. Stress levels were assessed on days 1, 2, 3, 5, 7, 9 and 12 according to the non-invasive Cat-Stress-Score (CSS). Body weights were measured on days 0, 7 and 12. Finally, adoption rates and length of stay (LOS) were determined.</p> <p>Major findings of the study are: (1) the mean Cat-Stress-Score decreased with time for all cats, but cats with a hiding box however showed a significant faster decrease in the CSS, reaching a lower CSS-steady state seven days earlier than the control group; (2) nearly all cats in both groups lost significant body weight during the first two weeks; (3) hiding boxes did not significantly influence weight loss; (4) no differences were found in the adoption rates and the LOS between both groups.</p> <p>Hiding enrichment reduces behavioural stress in shelter cats during quarantine situations and can therefore be a relatively simple aid to shelter adaptation. It offers no prevention however against feline weight loss, which indicates a serious health risk for shelter cats.</p>
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1 **The effect of a hiding box on stress levels and body weight** 2 **in Dutch shelter cats; a randomized controlled trial**

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26 **Abstract**

27 While staying in an animal shelter, cats may suffer from chronic stress which impairs their health and
28 welfare. Offering hiding opportunities can significantly reduce behavioural stress in cats, but
29 confirmation with physical parameters is needed. Therefore, the aim of this study was to determine the
30 effect of a hiding box on behavioural stress levels (scored by means of the Cat-Stress-Score) and a
31 physical parameter as body weight in newly arrived cats in a Dutch animal shelter during the first 12
32 days in quarantine situations.

33 Twenty three cats between 1 and 10 years of age were randomly divided between the experimental (N
34 = 12) and control group (N = 11) with and without a hiding box. Stress levels were assessed on days 1,
35 2, 3, 5, 7, 9 and 12 according to the non-invasive Cat-Stress-Score (CSS). Body weights were measured
36 on days 0, 7 and 12. Finally, adoption rates and length of stay (LOS) were determined.

37 Major findings of the study are: (1) the mean Cat-Stress-Score decreased with time for all cats, but cats
38 with a hiding box however showed a significant faster decrease in the CSS, reaching a lower CSS-steady
39 state seven days earlier than the control group; (2) nearly all cats in both groups lost significant body
40 weight during the first two weeks; (3) hiding boxes did not significantly influence weight loss; (4) no
41 differences were found in the adoption rates and the LOS between both groups.

42 Hiding enrichment reduces behavioural stress in shelter cats during quarantine situations and can
43 therefore be a relatively simple aid to shelter adaptation. It offers no prevention however against feline
44 weight loss, which indicates a serious health risk for shelter cats.

45

46 **Introduction**

47 About 200 animal shelters in the Netherlands take in and rehome 27.000 stray and relinquished cats
48 annually [1]. A shelter life is often associated with many stressors. Cats entering a shelter are introduced
49 to a foreign environment with unfamiliar animals, people, sounds and smells. During these first days
50 many of these cats struggle to adapt to these prolonged or repeated stressors and thus show stress
51 responses [2-4]. This may elicit clinical signs like hiding behaviour, defecating and urinating outside
52 the litter box, decreased grooming or over-grooming behaviour and a loss of appetite [2,5-8]. Stress-
53 induced longterm high cortisol levels can reduce the efficacy of the immune system against infectious
54 diseases [1,5,6,8,9], and chronic stress can therefore harm a cat's health as well [5,7,10,11].

55 When in a state of stress, the majority of cats will stop eating. Tanaka et al. found that stress elicited a
56 decrease in food intake, negatively correlated with stress scores [12,13]. This stress response can have
57 grave impact on cats: severe body weight losses in only a short period of time can induce feline hepatic
58 lipidosi [5,14,15].

59 Several studies show that stressed cats display increased alert resting behaviour behind their litter box
60 in an environment without hiding opportunities [10,16,17]. This is interpreted as alternative hiding
61 behaviour for it offers some concealment [10,16]. Real concealment can be offered by providing a hiding
62 box to shelter cats. A study of Kry and Casey [17] demonstrated a decrease in stress, measured by the
63 Cat-Stress-Score (CSS), when shelter cats were offered hiding boxes. Weight loss during quarantine is
64 another phenomenon in shelter cats associated with stress [12]. However, little research has been done
65 on the preventive effect of a hiding box on this stress induced weight loss.

66 A previous study conducted by Vinke et al. has been the first step to scientific evidence about the effect
67 of a hiding box on stress levels of newly arrived cats in a Dutch animal shelter during the first 14 days
68 in quarantine situations. The results show that cats with a hiding box recovered at least 4 days earlier
69 from stress than cats without a hiding box [10]. The present study was designed with more frequent CSS
70 scoring between day 5 and 12, to gain more insight in the feline recovery to stress and to relate these
71 behavioural stress levels to a physical parameter as body weight.

72 The primary aim of this study was to determine the effect of a hiding box on behavioural stress levels
73 and on body weight of newly arrived cats in a Dutch animal shelter during the first 12 days in quarantine.
74 The additional aim was to compare the Length of Stay (LOS) of cats in both study groups. It was
75 hypothesized that a hiding box would significantly reduce stress levels of newly arrived cats compared
76 to the non-hiding box group, reflected in a lower CSS, less weight loss and a shorter LOS.

77

78 **Materials and methods**

79 The study was approved by the Animal Welfare Body Utrecht, after assessing the present study. It was
80 concluded that the study does not meet the definition of an animal experiment as defined in the Dutch
81 Experiments on Animals Act and Directive 2010/63/EU because the animals encountered no discomfort.

82

83 **Animal shelter**

84 This study was performed at a Dutch animal shelter (Stichting Dierentehuis Arnhem en omstreken), a
85 medium size animal shelter with an open intake of around 700 cats per year [31]. Cat housing is situated
86 in five separate quarantine units, an isolation ward and an adoption unit, providing a maximum shelter
87 capacity of 90 cats in total. Dutch legislation mandates animal shelters to have quarantine and isolation
88 wards and a legal stray holding period of 14 days. New animals were quarantined at intake for at least 2
89 weeks, as is legally required [19]. For this study an informed consent was obtained from the shelter staff.

90 In order to relate this study to daily shelter management, the original shelter protocols about the intake
91 of new animals, daily animal care and hygiene were generally accepted, and substantial adjustments
92 were avoided.

93

94 **Animals**

95 For this study 23 European short hair cats, 11 males and 12 females, were selected out of the cats entering
96 the shelter between the 4th of November and the 30th of December 2015. At intake cats entering the
97 shelter were examined by the shelter staff for gender, breed and age and received a treatment against

98 ecto- and endoparasites (Stronghold[®] and Milbemax[®]). As all the cats came in as strays, age was
99 estimated in years. Within 5 days after intake the shelter veterinarian performed a physical health check.
100 During this veterinary check (during the morning hours) the cats were microchipped and vaccinated
101 with an attenuated vaccin (Versifel CVR[®]) against feline panleukopenia virus (FPV), feline herpes virus
102 (FHV-1) and feline calici virus (FCV). Intact cats were spayed or neutered after Day 14.
103 Inclusion criteria for this study were based on breed (European shorthair cats), health status and age
104 (between 1 and 10 years of age). When new cats showed no clinical signs of illness, obvious heat,
105 pregnancy or signs of nursing during the physical examination at intake, they were included in this
106 study. As it is not generally accepted practice in Dutch animal shelters to screen apparently healthy cats
107 through diagnostic testing (e.g. FIV/FeLV) at shelter intake, apart from the physical examination, no
108 additional information was available on the feline health status of the cats in this study.
109 All cats were observed for at least 12 days after intake.
110 During the study two cats participating in this study, left the shelter before their last observation day:
111 from the Hiding box group one cat went to a foster home, from the Control group one cat was released
112 within a trap-neuter-relaese (TNR) program. Data of both cats were excluded from this study. Two other
113 cats were not included in data for the length of stay, but were included in data for the Cat-Stress-Score,
114 body weight and the adoption rate. After the 12 days observation period, one of these cats (nr. 8, control
115 group) proved to be infected with FeLV and was euthanized a few days after the quarantine period of
116 14 days, while another cat (nr. 19, control group), because of its semi feral behaviour, was also released
117 through the TNR program. Because shelters often take in these non-clinical but infected cats and stray
118 cats being poorly socialized (and even rehome them), this study has included these two cats in three of
119 the four measured parameters.
120 The reason for selecting cats between 1 and 10 years of age was to avoid inclusion of juveniles with
121 related specific behaviour and elderly cats with increased chances for subclinical disease which might
122 influence their behaviour [18,20]. As previous studies [17,18] found no gender related significant
123 differences in stress behaviour, both male and female cats were included in the present study. The 23
124 cats were randomly assigned to one of the two groups with and without access to a hiding box.
125

126 **Housing conditions**

127 The cat housing in the two adjacent quarantine wards consisted of cages (L x W x H: either 84 x 95 x
128 80 cm or 69 x 91 x 87 cm) in which the cats were individually housed. Every cage was furnished with
129 a food and water bowl, bedding of towels, a litter box and a perching shelf at 28 cm above the cage
130 floor. The cages of the experimental group contained a hiding box that was placed at the right side at
131 the back of the cage. To avoid place preference for towels as bedding, the towels were covering the
132 entire floor of the cage including the shelf and the inside of the hiding box.

133 Cardboard boxes were used as hiding box and measured 44 x 31 x 26 cm (L x W x H). These boxes had
134 two entrances (WxH 0.16 x 0.20 m) [10]. Hiding boxes were never reused.

135 Access to the cats in the quarantine wards was restricted to the caretakers and the observer. Natural
136 daylight was provided through windows in both quarantine wards, combined with fluorescent lighting
137 between 08:00 AM. and 5:00 PM. Daily temperatures in the quarantine wards ranged from 16.0 to
138 19.8 °C. In the quarantine wards no dog vocalizations could be heard.

139

140 **Daily animal care**

141 The shelter staff cleaned the cages daily between 09:00 and 12:15 AM by removing waste and applying
142 a spot cleaning method [21]. During this procedure cats remained in their cages. Litter boxes were daily
143 cleaned with hot water and dried with clean paper towels. Cages were disinfected between cats or when
144 indicated (e.g. diarrhea) with a chlorine disinfectant containing sodium dichloroisocyanurate (Halacid®).

145 Food was provided once daily between 9:30 and 10:00 AM and comprised of around 50g per day Adult
146 Royal Canin® dry cat food (SC 365D) with a metabolizable energy content (ME) of 4066 kcal/kg (16.995
147 MJ/kg). Fresh water was provided ad libitum. Cats kept their own litter box for the time of this study.

148

149 **Behavioural observations**

150 Cats were given an habituation period of 24 hours after shelter intake (= Day 0), before behavioural
151 assessment was performed [17]. Behavioural data were collected on days 1, 2, 3, 5, 7, 9 and 12 between
152 12:30 and 5:15 PM, during which interactions with caretakers were avoided.

153 Each cat was observed for 20 minutes per day by using video-recording. Outside the cage a video camera
154 (H.264 DVR) was mounted on a tripod at cage height. For new observations the combination camera-
155 tripod had to be readjusted to the new cat cage. Video recordings were viewed in real-time in an adjacent
156 room and stored for subsequent analysis (Fig 1). Only one camera was used for recording.

157

158 **Fig 1. Diagram of the experimental set up, observer and both camera positions in the quarantine**
159 **wards in the animal shelter.**

160

161 **Cat-Stress-Score (CSS)**

162 Kessler and Turner [18,22] developed a 7-level cat stress score (CSS) which has been used in several
163 studies to estimate stress levels in confined cats [3,10,17,18,]. This scoring system assesses the level of
164 feline stress based on the posture of body elements (e.g. belly, legs, tail, head, eyes, pupils, ears,
165 whiskers) and behaviour (vocalization and activity) as described in the ethogram of the UK Cat
166 Behaviour Working Group [22]. The CSS ranges from 1 (fully relaxed) to 7 (terrorized).

167 One observer (LS) assessed the CSS score per cat on Day 1, 2, 3, 5, 7, 9 and 12. Intra-observer
168 variation was minimized by observational training using (video) images of pre-described feline
169 behaviours from previous experiments with shelter cats.

170 After the video camera had been positioned, the scan sampling started after 2 minutes in which the cat
171 habituated to the novel situation. Thereafter the cat was scored according to the Scan Sampling method,
172 in which four scores (= four samplings) were made during the observation time (the 1st observation at 5
173 min, the 2nd at 10 min, the 3rd at 15 min and the 4th at 20 min) [23]. Imperceptible posture and behavioural
174 elements were noted as missing values.

175 Each of the elements of the Cat-Stress-Scores was scored separately. The scores of the four samplings
176 were averaged to assign an overall CSS for each separate cat per day.

177

178 **Body weight**

179 During the study every cat was weighted on Day 0, 7 and 12 by using an electronic scale^a (accuracy \pm
180 10 g). The standardized shelter feeding regime consisted of approximately 50g per cat per day of Adult
181 Royal Canin (RC)[®] dry cat food, which equals 203.3 kcal or 849.8 kJ per cat per day.
182 To secure adequate nutrition for the cats in this study, the daily caloric feline requirements (FEDIAF
183 guidelines (80 kcal [335 kJ] ME per kg^{0.67})) were determined per individual cat [24].

184

185 **Adoption rates and length of stay (LOS)**

186 In order to determine the effect of a hiding box in quarantine situations on the subsequent adoption
187 success, the adoption dates of the cats in this study were noted. Adoption rates (= # cats adopted / all
188 cats in this study) and the length of stay (LOS: number of days between the shelter intake of a cat and
189 its day of adoption) was determined per cat. The LOS included the mandatory quarantine period of two
190 weeks and only included adopted cats, excluding cats which were euthanized or returned to their outdoor
191 environment after finishing this study.

192

193 **Statistical analyses**

194 A randomised controlled trial (RCT) design was used [25]. Data were stored in Microsoft Excel 2010
195 files (Microsoft Corp, Redmond, Wash.). Two statistical software programs were used for analysis of
196 the data:

197 - SPSS (IBM Corp, Armonk, NY version 25) for the two-sample T-test and chi-square test.

198 - R (version 3.3.0) for the linear mixed regression models [28].

199 For the statistical analysis of effect of time and hiding box on the CSS (model ‘CSS-Time-Box’) a linear
200 mixed regression model [27] was assumed, with the CSS as the outcome, while Time after arrival, the
201 availability of a hiding box and the interaction between both were used as explanatory factors. CatID
202 was used as the random effect to take the correlation between observations within cat into account. An
203 AR1 correlation between the time points was added as well as a variance model to allow different
204 variances for the separate time points. A maximum likelihood-based method was used to calculate the

205 Akaike's Information Criterion (AIC) to select the best model using a backward selection approach
206 (smaller is better).

207 For the statistical analysis of effect of time and hiding box on the body weight, a linear mixed regression
208 model [27] was used to analyse the weight as the outcome and Time after arrival, the availability of a
209 hiding box and the interaction between both as explanatory factors. Although keeping the box in the
210 linear mixed model resulted in a worse fit of the model, the availability of the box nevertheless was
211 added in coherence with our primary aim. Also in this model CatID was used for the random effect.

212 The validity of both models was confirmed by a visual inspection of the residuals for normality and
213 constance of variance.

214 Per experimental group the number of adopted cats was analyzed using chi-square test, while the length
215 of stay (LOS) was analyzed using the two-sample T-test. The assumptions for these variables for equal
216 variance (Levene's test) and for normal distribution (Shapiro-Wilk's test) were met.

217 We reported the estimated effects of the availability of a hiding box according the reporting guidelines
218 for randomized controlled trials (www.reflect-statement.org).

219

220 **Results**

221

222 **Characteristics of the study population**

223 The experimental group consisted of 12 cats (6 males and 6 females) of which the estimated age ranged
224 between 1 and 7 years (mean: 3.3 years, SD: 2.2). The control group consisted of 11 cats (5 males and
225 6 females) with estimated ages between 1 to 10 years (mean: 4.9 years, SD: 3.1, with n=10: due to her
226 semi feral behaviour no age could be estimated of cat nr 19).

227 The cats in this study are presented in the appendix with their ID, experimental group, gender, age,
228 bodyweight at intake (kg) and the quarantine wards they went after intake.

229

230 **Daily Cat-Stress-Score (CSS): behavioural assessment**

231 The time-dependent reduction of the individual CSS per cat in both groups is visualized in Fig 2.

232

233 **Fig 2. Course of the Cat-Stress-Score in time of individual cats from the control group and the**
234 **experimental group.**

235

236 Cats from the hiding box group reached a steady state sooner (at day 2) than cats from the control group
237 (at day 9). The model results for the mean CSS are presented in Table 1. The estimated means of the
238 CSS of the hiding box group (mean CSS = 2.7) and the control (mean CSS = 3.1) at Day 1 are similar
239 as their difference is not significant (-0.4, 95% CI:-0.97 to +0.12). At all other days the mean CSS of
240 the hiding box group is significantly lower than the mean CSS in the control group, largest at day 2 (-
241 0.99, 95%CI: -1.38 to -0.61) and decreasing in difference between the groups on day 12 (-0.33, 95%CI:
242 -0.57 to -0.08).

243

244 **Table 1. Results of the model for the Cat-Stress-Score with 95% confidence interval,**
245 **influenced by Day and availability of a hiding box and interaction between both.**

Research group	Time (days after Intake)	Estimate	Lower bound 95% CI ¹	Upper bound 95% CI ¹
Control	Day 1	3.13 ²	2.74	3.53
Control	Day 2	-0.11 ³	-0.43	0.20
Control	Day 3	-0.54 ³	-0.91	-0.17
Control	Day 5	-0.76 ³	-1.15	-0.37
Control	Day 7	-0.82 ³	-1.21	-0.42
Control	Day 9	-0.92 ³	-1.32	-0.53
Control	Day 12	-0.91 ³	-1.34	-0.49
Hiding box	Day 1	-0.43 ⁴	-0.97	0.12
Hiding box	Day 2	-0.99 ⁴	-1.38	-0.61
Hiding box	Day 3	-0.51 ⁴	-0.79	-0.23
Hiding box	Day 5	-0.25 ⁴	-0.47	-0.03
Hiding box	Day 7	-0.23 ⁴	-0.40	-0.05
Hiding box	Day 9	-0.12 ⁴	-0.24	-0.01
Hiding box	Day 12	-0.33 ⁴	-0.57	-0.08

246 Legend:

247 ¹ CI = Confidence Interval

248 ² Mean CSS in cats in Control group at day 1.

249 ³ Difference between mean CSS at specified day in Control group compared to mean CSS at day 1 of
250 same cats.

251 ⁴ Difference between mean CSS at specified day in cats of group with Hiding box compared to mean
252 CSS of cats in group Control group at same day.

253

254 **Body weight**

255 For the comparison of both experimental groups, the absolute body weight was used. At intake the
256 control cats were on average 300 grams heavier than those in the experimental group. This initial weight
257 difference between both groups reduced to 210 grams at Day 7 and Day 12: cats in the control group
258 lost overall 7.7% of their initial body weight, while cats with a hiding box lost 6.3% of their initial body
259 weight during those 12 days (Table 2). The initial weight and weight reduction between the groups
260 however proved not to be significant.

261

262 **Table 2. Results of the model for Body weight with a 95% confidence interval, influenced by Day**
263 **and availability of a hiding box and interaction between both.**

Research group	Time (days after Intake)	Estimate (kg)	Lower bound 95% CI ¹ (kg)	Upper bound 95% CI ¹ (kg)
Control	Day 0	4,39 ²	3,77	5,01
Control	Day 7	-0,25 ³	-0,35	-0,15
Control	Day 12	-0,32 ³	-0,42	-0,22
Hiding box	Day 0	-0,30 ⁴	-1,16	0,56
Hiding box	Day 7	-0,21 ⁴	-1,07	0,65
Hiding box	Day 12	-0,21 ⁴	-1,07	0,65

264 Legend:

265 ¹ CI = Confidence Interval

266 ² the mean Body weight of cats in the Control group at Day 0.

267 ³ Difference between the mean Body weight of cats in the Control group at the specified Day
268 compared to the mean Body weight of the same cats at Day 0.

269 ⁴ Difference between the mean Body weight of cats in the Hiding box group and the mean body
270 weight of cats in the Control group at the specified Day.

271

272 The individual proportional decrease in body weight is visualized in Fig 3. All cats except one lost
273 weight during both weeks. When weight loss at Day 12 was calculated as a percentage of initial body
274 weight at intake, it was found that 7 of the 23 (35%) cats lost $\leq 5\%$ of their body weight, whereas 15 of
275 the 23 (65%) cats lost 5% or more of their weight. The maximum body weight loss was found in cat nr.
276 8 (control group) which lost 19% of its initial weight in 12 days and was diagnosed with an infection of
277 FeLV a few days after completing this study.

278

279 **Fig 3. The proportional change (%) in body weight in individual cats from the control group and**
280 **the experimental group.**

281

282 **Adoption rates and length of stay (LOS)**

283 Of the 23 shelter cats in this study, 21 were rehomed after the observation period was finished. In the
284 control group 9 out of 11 cats were adopted (82%), in the experimental group 12 out of 12 (100%). No
285 significant difference was found in the adoption rate between the two groups ($p = 0.55$).

286 As we defined LOS as the number of days between the shelter intake of a cat and its day of adoption, 2
287 cats were not included in this data set, for they were not adopted.

288 The mean LOS for the control group ($n = 9$) was 24.1 days (SD 5.4, range 15-30 days) and for the hiding
289 box group ($n = 12$) was 22.9 days (SD = 4.4, range 16-30 days). No difference in the mean LOS was
290 found between control and the hiding box group (p -value = 0.58).

291

292 **Discussion**

293 The aim of the present study was to determine the effect of a hiding box on behavioural stress levels
294 and body weight in shelter cats during the first 12 days in quarantine. While in a previous study cats
295 were monitored on days 1 through 5 and the 14th day [10], this new study added more insight about the
296 differences in CSS between Day 5 and Day 12.

297 The most important findings of this study are:

- 298 – The mean Cat-Stress-Score decreased with time for all cats, but cats with a hiding box however
299 showed a significant faster decrease in the CSS and recovered from stress seven days earlier
300 than the control group.
- 301 – Nearly all cats lost significant body weight during the first two weeks. On average, cats with
302 hiding boxes lost 40 grams less of their initial body weight compared with cats without a box,
303 although this difference was not significant.
- 304 – The mean adoption rates and the LOS of cats with and without hiding boxes were equal.

305

306 **Cat-Stress-Score (CSS): behavioural assessment validation**

307 In this study, cats with a hiding box showed a significant faster decrease of behavioral stress compared
308 to the control group, which was most prominent during the first observation days. These results were in
309 line with earlier findings of Vinke et al. [10] and with a study of Gourkow and Fraser, in which the mean
310 CSS of cats, housed in single barren cages without positive human-cat interaction, was higher compared
311 to the other groups and only reached a similar CSS on Day 9 [13].

312 The findings of the present study complete the results obtained by Vinke et al., where the hiding box
313 group recovered at least four days earlier. By increasing the number of observational days during the
314 first 12 days, the current research provides more details in reaching the CSS-steady state, indicating that
315 hiding boxes accelerate the recovery of behavioral stress by seven days. This is important, because the
316 hiding box clearly helps the shelter cat to adapt more quickly in a stressful new environment. This in
317 order to prevent the development of chronic stress [17].

318

319 **Body weight**

320 This study shows a significant decrease in feline body weight during the first 12 days in an animal
321 shelter. Approximately a third of the cats lost less than 5% body weight during the first 12 days, while
322 two-third lost over 5%. These results agree with previous findings of Tanaka et al., in which 57% of
323 their cat population showed 5% or more weight loss during their shelter stay [12].

324 When otherwise healthy cats lose weight unintentionally, it is a dramatic indicator of a health risk.

325 Weight loss can be caused by insufficient nutritional management (the shelter offers inadequate quantity
326 and/or quality of food) and also by a decrease in feline appetite by a physical stress response. Although
327 food intake was not registered in the present study, it was observed that some cats were completely
328 anorectic, especially during the first days. For the shelter this was the reason to standardize the feeding
329 schedule of 50 g dry cat food per cat per day. According to the FEDIAF guidelines [24] for daily caloric
330 feline requirements, during this study cats over 4.01 kg might have been offered an inadequate amount
331 of food. With an individual requirement of 80 kcal (335 kJ) ME per kg^{0.67}, 50 g dry cat food per day will

332 meet maintenance energy requirements of cats up to a body weight of 4.01 kg. Cats weighing over 4.01
333 kg, need more Adult RC food daily. Of the 23 cats, 13 (57%) cats weighed more than 4.01 kg. The
334 heaviest cat weighed 6.41 kg at intake and hence required at least 68.4 grams of catfood per day. During
335 the daily observation however, cats rarely finished their food rations during these first two weeks. An
336 inadequate quantity of food was therefore not considered to be the cause of the observed body weight
337 loss.

338 The effect of stress however on the body weight of shelter cats was first shown by Tanaka et al. [12],
339 who found a negative correlation between food intake and stress scores of cats. The conclusion was that
340 cats, admitted to an animal shelter were likely to lose weight while in the shelter. These results are
341 consistent with our findings, indicating that a decrease of feline appetite caused by a physical stress
342 response, is most likely responsible for the weight loss.

343 Although the provided commercial food in this study was of a high quality, there is less understanding
344 of the role of palatability of food for shelter cats in relation to weight loss. The only cat in this study
345 which gained weight, received medication for cystitis (meloxicam 0.05 mg/kg) mixed with canned food.
346 This gives an indication of the importance of palatability of food for shelter cats.

347 Although the analysis of the effect of time and the presence of a hiding box on the body weight suggested
348 that there was a difference between the two groups in body weight losses, as cats with hiding boxes
349 showed approximately 40 grams less weight loss in comparison with the control group, this difference
350 was not significant. For the individual cat, however, this could be biologically relevant, for weight loss
351 due to feline anorexia has a serious impact on a cat's health, increasing the risks of hepatic steatosis
352 [5,14,15]. A significant difference in body weight between cats with and without hiding facilities,
353 however, was not identified. Therefore more research is necessary to monitor these cats for a longer
354 period of time, to register the process of adaptation to the new environment in correlation to the weight
355 losses and to experiment with ways to prevent or reduce body weight losses in shelter cats (i.e. highly
356 valued palatable food items might overcome the fear motivation and stimulate consumption despite the
357 challenging environment).

358 Apart from stress, progressive weight loss can also be a sign of serious medical problems [32]. One of
359 the cats from the control group showed a weight loss close to 20% in 12 days and was eventually

360 diagnosed with FeLV. Shelters could use weight loss during quarantine time as an early warning sign
361 for serious declines in physical conditions, but this asks for a daily weighing as a standard procedure.
362

363 **Adoption rates and length of stay (LOS)**

364 Sometimes shelter staff expresses their worries about using hiding boxes, for boxes might decrease the
365 visibility of cats to potential adopters and therefore slow down adoption rates (personal
366 communications). Kry and Casey however showed that providing shelter cats with hiding enrichment,
367 did not decrease the likelihood of those cats being adopted [17]. Also our study did not reveal differences
368 in the adoption rates and the LOS of both groups. The hiding enrichment itself, however, could not have
369 influenced the adopters' choices based on the (in)visibility of the cat, for hiding boxes were only present
370 in 12 of the 23 cages during the first 12 days of quarantine time, while no hiding boxes were available
371 in the adoption area.

372

373 **Finally, Stressors versus Signals of Safety?**

374 Historically, the science of stress emphasizes the role of stressors in evoking stress responses. Stress
375 reduction is caused by reducing the impact of a stressor (in number, strength, duration, etc) on the
376 behavioural and physiological responses of the individual in question by adapting to it [30]. Stressed
377 cats are likely to reduce their feed intake and subsequently loose body weight. Cats in shelters [12], in
378 laboratories [16], in boarding facilities and even privately owned cats [5] show this general stress
379 response to stressors in challenging situations. Because previous research had shown a robust effect of
380 the hiding box on the behavioural stress response [10] reflecting adaption, expectations were that hiding
381 opportunities would also aid in the reduction of weight loss. While this study proved again a significant
382 decrease of the behavioural stress response when shelter cats were offered a hiding opportunity, the
383 effects on body weight were minor. These results challenge our point of focus on stress in shelter
384 animals: a shelter environment offers numerous stressors for which feline hiding behaviour appears not
385 sufficient enough to induce a fast adaptation, for more than 90% of the cats with and without hiding
386 opportunities lost body weight during the first 12 days after intake. New theories on human stress

387 response mechanisms might shed some light on the feline stress response in these complex shelter
388 environments and contribute to more practical tools for stress reduction. According to Brosschot [33],
389 who introduced the Generalized Unsafety Theory of Stress (GUTS), ‘the stress response of the body is
390 always “on” and it stays on as long as there is no obvious safety.’ This default response can only be
391 inhibited when ‘signals of safety’ are perceived by the animal. We therefore should not look for the
392 causation of a stress response but rather ask ourselves ‘what stops the stress response?’. When present
393 results are reviewed in the light of this GUTS, the hiding enrichment itself caused a decrease in feline
394 behavioural stress scores, but did not provide an adequate signal of safety (SOS) to prevent weight loss
395 in most cats. Changing the food presentation (e.g. food offered inside hiding boxes) might give shelter
396 cats more safety signals. This GUTS approach asks for a comparison of the effect of distinct SOS’s (like
397 hiding materials, food presentations, enriched feeding, feline pheromones, human contact, increased
398 cage space, solitary housing, etc) and for the reinforcing effects of combining these signals on the
399 majority of shelter cats. In addition to focusing on reduction of numerous stressors in the shelter
400 environment, we should also search for signals of safety which are strong enough to inhibit the stress
401 response and thus create a situation which the majority of animals can perceive as safe.

402

403 **Limitations**

404 Limitations of this randomized controlled trial included the small sample size.

405

406 **Conclusion**

407 Providing hiding boxes can be a relatively simple way for cats to self-manage stress and to adapt faster
408 to the shelter environment. The majority of the shelter cats however loose (considerable) weight during
409 the quarantine time in an animal shelter. Providing them with hiding enrichment during that period,
410 gives no prevention against this weight loss. Neither do hiding boxes have effect on the adoption rates
411 and the length of stay of both groups.

412 However, instead of keeping focus on identifying and reducing stressors in a very challenging
413 environment like an animal shelter, an additional approach could be found in the application of ‘signals

414 of safety' (SOS), strong enough to inhibit the stress response and thus create a situation which animals
415 can perceive as safe.

416

417

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424

425 **Author contributions**

426 Van der Leij conceived the study and Vinke assisted with the study design. Selman assisted with data
427 collection and data maintenance. Vernooij, Selman and Van der Leij analyzed the data and drafted the
428 manuscript. Vinke supervised the draft and submission.

429

430 **Conflicts of interest**

431 The authors declare that no conflict of interest exists in which any author or authors' institution has a
432 financial or other relationship with other people or organizations that may inappropriately influence the
433 authors' work.

434

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531
532

533 **Supporting information**

534 **S1 Fig 1. Diagram of the experimental set up, observer and both camera positions in the**
535 **quarantine wards in the animal shelter.**

536

537 **S2 Fig 2. Course of the Cat-Stress-Score in time of individual cats from the control group and the**
538 **experimental group.**

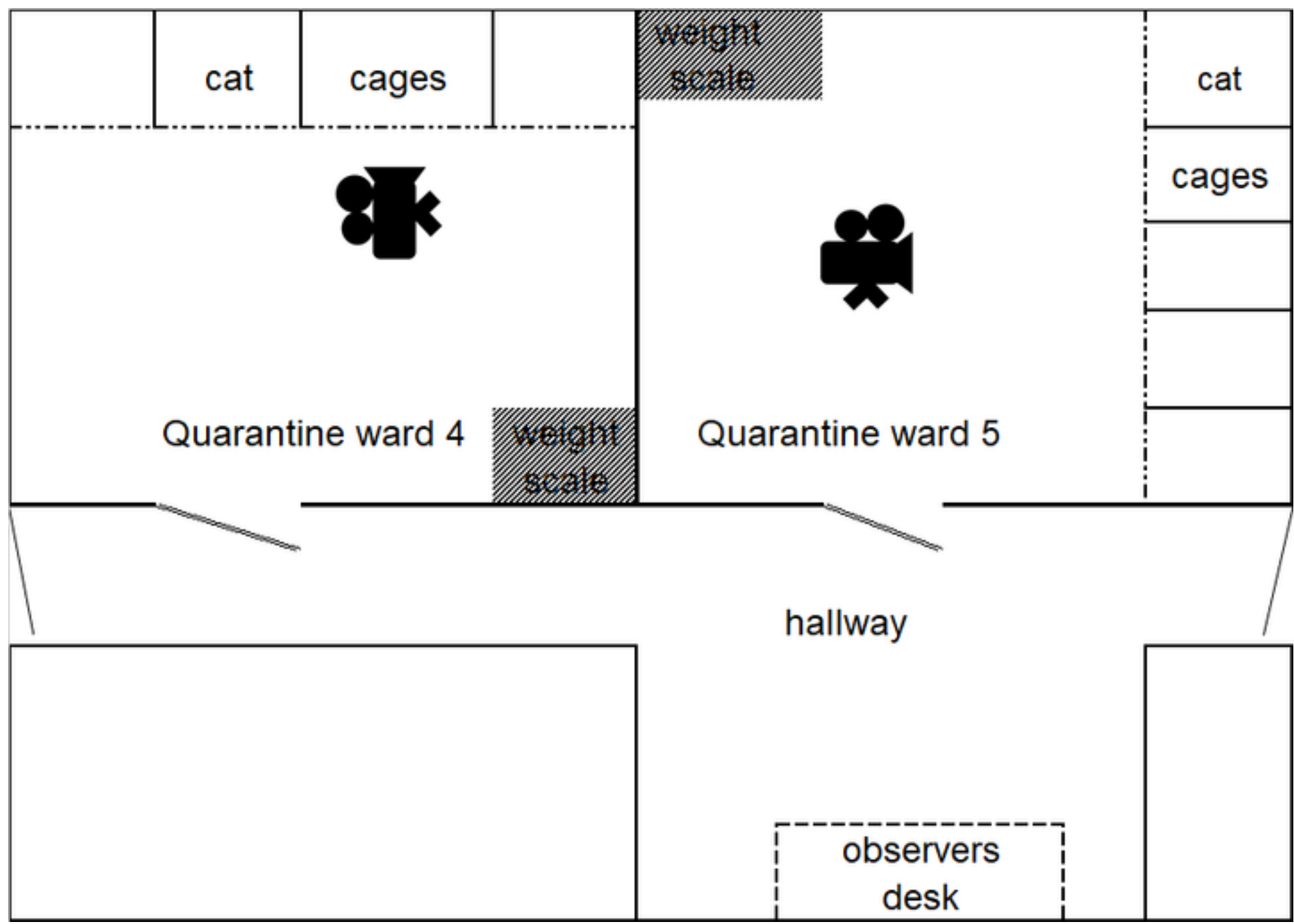
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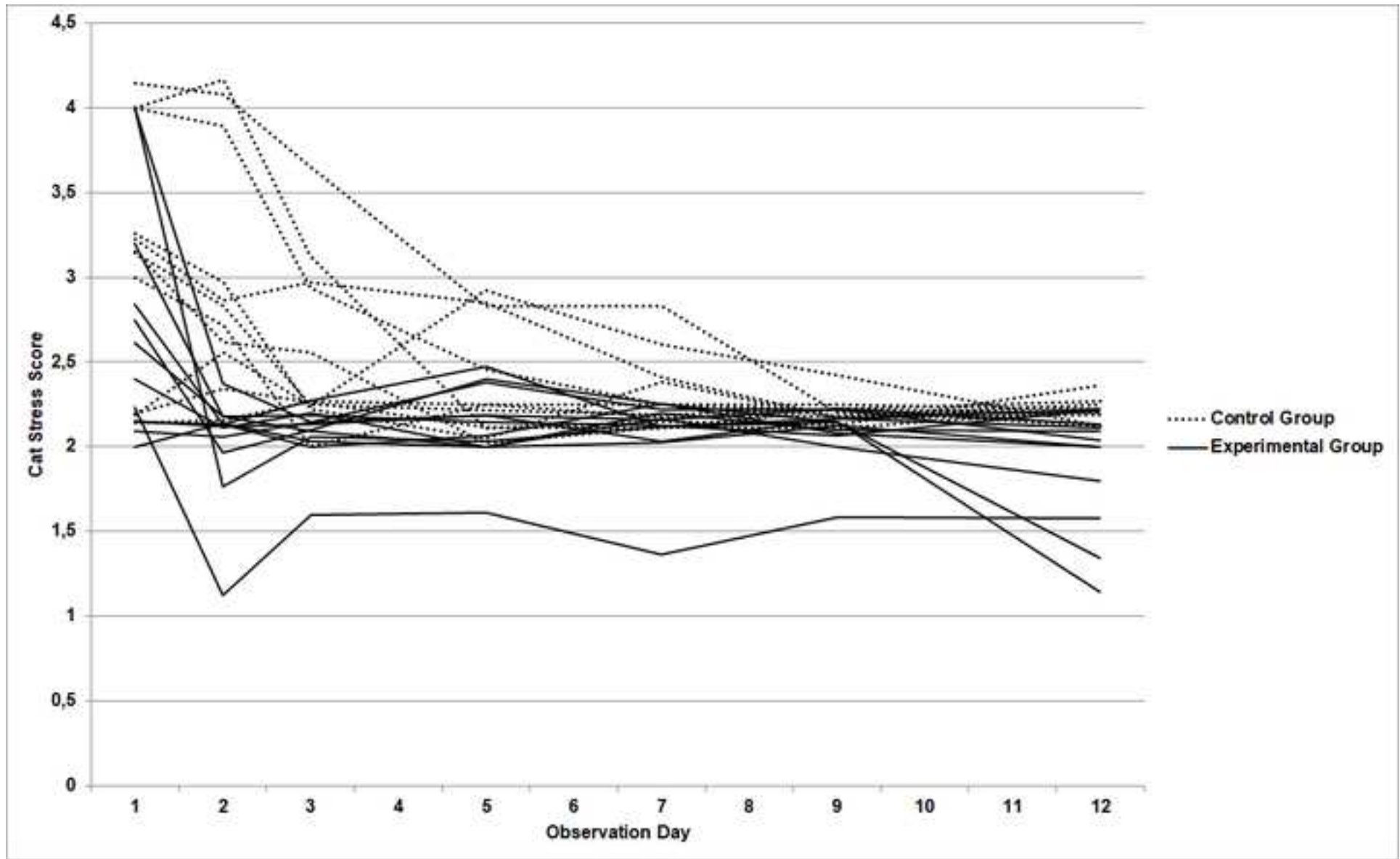
540 **S3 Fig 3. The proportional change (%) in body weight in individual cats from the control group**
541 **and the experimental group.**

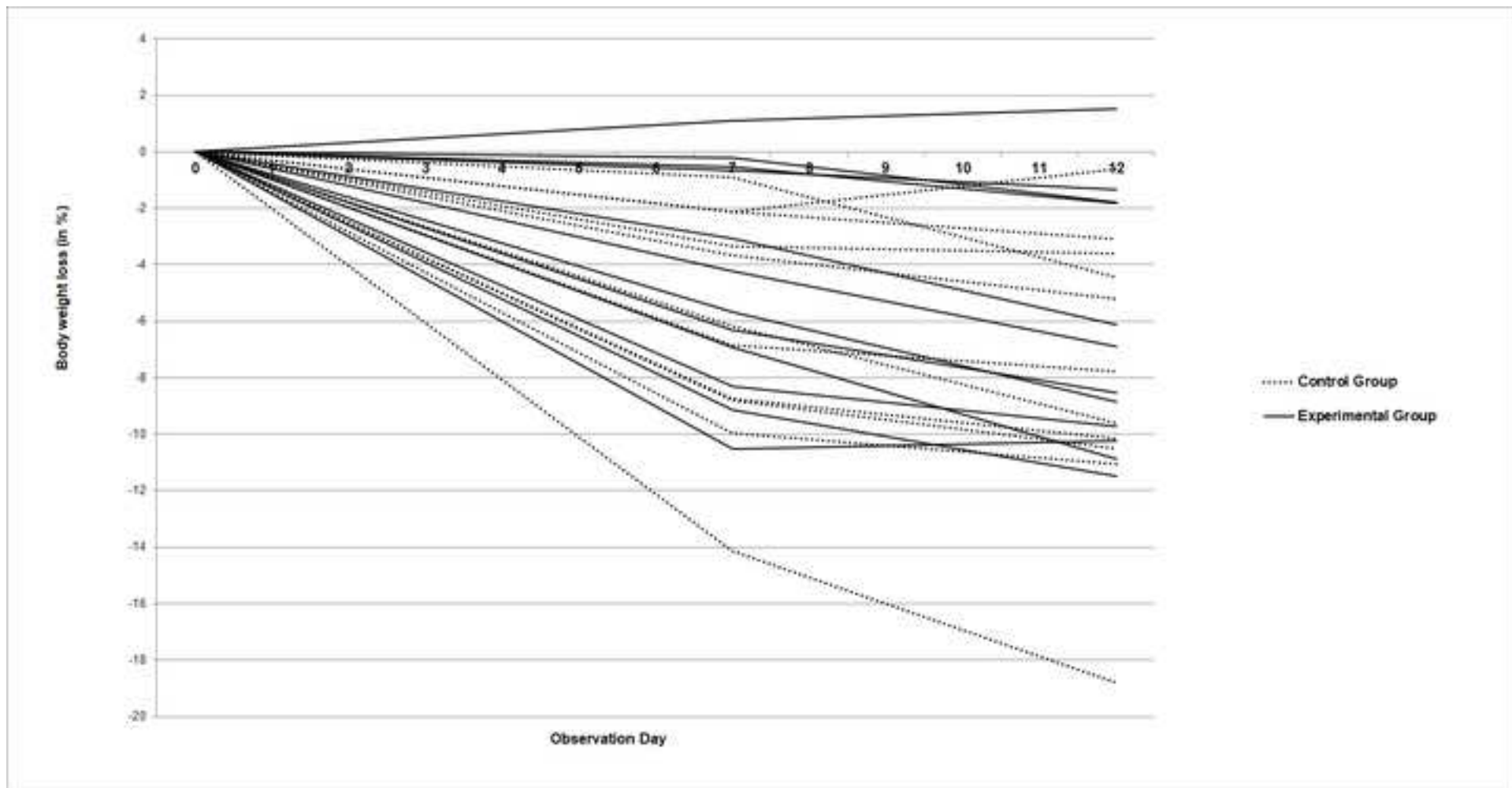
542

543 **S1 Appendix. Baseline characteristics of treatment cohorts in a randomized field trial**
544 **comparing quarantine cat housing with and without hiding opportunities in one Dutch animal**
545 **shelter.**

546









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Supporting Information
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