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Relation	



1	The effect of communal litter box provision on the defecation behavior of free-roaming
2	cats in old-town Onomichi, Japan
3	
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11 ABSTRACT

12	Feces littered on the ground by free-roaming cats contain bacteria, viruses, and parasites and
13	pose a significant health risk to humans. The purpose of this study was to examine the effect of
14	communal litter box provision on the defecation behavior of a free-roaming cat population. The
15	study was conducted at H temple and its graveyard in the uptown area of old-town Onomichi,
16	Japan. Cat feces were collected and weighed once a week for 4 weeks, at five popular
17	defecation sites in the temple precincts and graveyard, to assess the quantity of feces left by the
18	cats. A commercial cat repellent was then applied to the ground at 11 sites, including the five
19	popular defecation sites, and six communal litter boxes, created by filling repurposed plastic
20	planters with cat litter, were provided at different sites. The feces in the six litter boxes and on
21	the ground at the five defecation sites were collected and weighed once a week for 14 weeks.
22	The behavior of the cats around the litter boxes and defecation sites was captured using trail
23	cameras. The total weight of the feces collected from the ground before the application of the
24	litter boxes and cat repellent was 939 g. Three adult cats were mainly responsible for the feces
25	on the ground. The amount of feces found on the ground around the temple decreased gradually
26	and significantly after the provision application of the litter boxes and repellent, and reached 0 g

27	in the final week of the study. In contrast, the average weight of the feces in the six litter boxes
28	increased gradually and significantly, and reached 65.7 g/litter box/week in the 14th week. The
29	results showed that the provision of litter boxes and the use of repellent is effective in changing
30	the defecation behavior of ownerless free-roaming cats. We recommend promoting the
31	provision of litter boxes to free-roaming cats to reduce fecal pollution in Onomichi and
32	engaging with local cat feeders to participate in the management of the litter boxes, such as
33	cleaning and changing the litter.
34	Keywords: Free-roaming cats, Defecation behavior, Feces, Communal litter box
35	
36	1. Introduction
37	The contribution of free-roaming cats to fecal pollution has not received much attention. It
38	was, however, reported that the free-roaming cats living in three communities in California
39	contributed about 76.4 tons of feces to the environment annually (Dabritz et al., 2006). Cat feces
40	pose a significant threat to human health because of the presence of bacteria, viruses, and
41	parasites that can infect humans and their pets (Voslářvá and Passantino, 2012; Gerhold et al.,
42	2013). For instance, cats are hosts to zoonotic parasites, such as the protozoan, Toxoplasma

43	gondii, and the ascarid, Toxocara cati. Playgrounds, private gardens, and public parks
44	contaminated by cat feces can serve as sources of infection for humans (Lee at al., 2010).
45	Children can accidentally come into contact with T. cati eggs when they play in sandboxes
46	(Despommier, 2003).
47	As free-roaming cat populations are increasing in urban areas around the world, controlling
48	these populations is a pressing issue. The trap-neuter-release (TNR) program has recently been
49	accepted as a viable tool in managing cat populations. However, Natoli et al. (2006) concluded
50	that TNR programs alone are not sufficient for managing urban feral cat populations. In contrast,
51	Kilgour et al. (2017) proposed that the TNR program be continued over multiple years. They
52	suggested that controlling cat populations is a long-term project and immediate effects cannot
53	be expected. However, zoonotic diseases from cat feces greatly concern residents in urban areas,
54	and this problem should be handled without delay, while simultaneously attempting to control
55	the numbers of free-roaming cats. One possible solution is to provide communal litter boxes in
56	areas where cat defecation is frequent. However, there is no research showing that the provision
57	of litter boxes would change the behavior of free-roaming cats from defecating on the ground to
58	defecating in the provided litter boxes.

59	The objective of this study was to examine the effect of communal litter box provision on the
60	defecation behavior of a free-roaming cat population. Our hypothesis was that free-roaming cats
61	that defecated on the ground would change their behavior if a cat repellent was applied at sites
62	where cat defecation was not desired and litter boxes were provided where cat defecation was
63	preferred.
64	
65	2. Material and methods
66	2.1. Study area
67	The study was conducted at an H Buddhist temple and its attached graveyard (Fig. 1) with a
68	total area of 3,976 m ² . The temple is located in the uptown area of old-town Onomichi, Japan,
69	which is recognized as a "town of cats" where approximately 200 free-roaming cats live (Seo
70	and Tanida, 2018). The town consists of residential and tourist areas with many historic temples
71	and shrines. The city office of Onomichi receives complaints from the residents of the town
72	about cat feces soiling the paths and grass and reducing the air quality of the neighborhood. The
73	Hiroshima prefectural animal shelter financially supports the residents and temples in the old
74	town through a TNR program; however, the defecation behavior of the neutered cats returning

75	to their original territory should still be controlled. The temples and shrines serve as havens for
76	the cats because harming or killing living things conflicts with the Buddhist and Shinto
77	doctrine.
78	
79	2.2. Study procedure
80	The staff of the H temple identified five popular defecation sites for the free-roaming cats in
81	the temple premises and attached graveyard (Fig. 1). Cat feces were collected at these five sites.
82	Each piece of feces was collected with tweezers and weighed with a compact digital scale
83	(Digital kitchen scale EM3000-PI2, Takeda Corporation, Nagoya, Japan) once a week for 4
84	weeks to calculate the amount of feces left by the cats. The amount of cat urine was not
85	measured in this study. Four trail cameras (Ltl-Acorn, Ltl-6210MC, Ltl-6310MC; Zhuhai Ltl
86	Acorn Electronics Co., Ltd., Zhuhai, China) were set up at defecation sites 1 to 4 to identify
87	individual cats (Fig. 1). The cameras were triggered by movement and captured pictures and
88	videos automatically for 60 s when triggered. Setting a trail camera at site 5 was not possible
89	because the site was near a tourist trail where people often walk. The SD cards and batteries in
90	the trail cameras were replaced weekly.

91	After 4 weeks of weighing cat feces, cat repellent (Cat Repellent; Technology Research
92	Institute of Osaka Prefecture, Osaka, Japan) that primarily emitted the smell of acetic and
93	isovaleric acids, was applied at 11 sites (the five popular defecation sites in addition to six sites
94	where the monk of the temple did not want the cats to defecate) (Fig. 1). The effectiveness of
95	the cat repellent has been reported in previously by Seo and Tanida (2016, 2017).
96	Simultaneously, six roofed cat litter boxes, created from repurposed plastic planters and filled
97	with commercially available cat litter (Woody Fresh WF-70, IRIS OHYAMA, Sendai, Japan),
98	were placed at different sites (Fig. 1). The dimensions of the litter boxes were 18.5 \times 25.0 \times
99	65.0 cm (Fig. 2). The volume of each litter box was 19,761 cm ³ and they could hold 10 L of cat
100	litter. The four trail cameras were positioned so that they could capture the cats defecating on
101	the ground as well as using the litter boxes. The feces in the litter boxes and on the ground at the
102	popular defecation sites were collected and weighed every week for 14 weeks. The cat litter was
103	cleaned and new litter was added once a week.
104	

105 2.3. Control study

106 We selected three sites in the town where free-roaming cats had been constantly defecating on

107	the ground as the control sites. These were K park (1,561 m ²), U Shinto shrine (3,476 m ²), and P
108	small park (96 m ²). Communal litter boxes were not placed at these three sites. Feces were
109	collected and weighed every week for 18 weeks from three sites in K park, three sites in U
110	Shinto Shrine, and two sites in P small park. Observations using trail cameras were not
111	permitted in these areas.
112	
113	2.4. Statistical analysis
114	Kruskal–Wallis tests with Shirley–Williams multiple comparisons were used to test weekly
115	changes in the numbers of defecation events and the weights of the feces in the litter boxes and
116	on the ground. The statistical package, Ekuseru-Tokei 2012 (Social Survey Research
117	Information Co., Ltd., Tokyo, Japan), was used to conduct these tests.
118	
119	3. Results
120	3.1. Defecation behavior before the provision of communal litter boxes
121	Seventeen cats were identified on the temple premises from the camera footage and human

122 observation. Cat feeders, who were either local caretakers or tourists, were observed on the

123	temple premises and in the surrounding neighborhood. Most of the cats were dependent on the
124	food they supplied. Using the camera data, we confirmed that three of the 17 cats (cats A, B,
125	and C) were responsible for defecating on the ground at the four sites with cameras (site 5 had
126	no camera) during the 4 weeks prior to providing the litter boxes. Thus, we focused on the
127	behavior of cats A, B, and C in this study. The three cats were tamed female feral cats. Before
128	providing the litter boxes, the total weight of feces on the ground at the five popular defecation
129	sites over the 4 weeks was 939 g (78.3 g/cat/week). Almost all the feces were dry when
130	collected.
131	
132	3.2. Defecation behavior after the provision of communal litter boxes
133	The weekly changes in the number of defecation events in the litter boxes by cats A, B, and
134	C is presented in Fig. 3. All three cats started to use the litter boxes in the first week after they
135	were provided, but rarely used the boxes from the third to sixth week because of the bad
136	weather during that period. The weekly number of defecation events in the litter boxes increased
137	over time but this change was not statistically significant.

138 The weekly changes in the number of defecation events on the ground at the four popular

139	defecation sites by cats A, B, and C before and after the litter boxes were provided is presented
140	in Fig. 4. The weekly number of defecation events on the ground by the three cats decreased
141	significantly (Kruskal–Wallis chi squared = 25.28, df = 14, $P = 0.0319$) after the litter boxes
142	were provided. The defecation rates in the litter boxes (the number of defecation events in the
143	litter boxes/the total number of defecation events) of cats A, B, and C were 81.3%, 88.6%, and
144	100%, respectively.
145	The number of defecation events by cats A, B, and C in each litter box during the 14-week
146	experimental period is shown in Fig. 5a. Litter box 3 was heavily utilized by all the three cats.
147	Litter boxes 1, 2, and 3 were mainly used by cat B, whereas litter boxes 4, 5, and 6 were mainly
148	used by cat C. The use of litter boxes by cats B and C mainly occurred between 6.00 h and
149	11.00 h, whereas cat A defecated randomly (Fig. 5b).
150	The weekly weight of feces (g/litter box/week) in the six litter boxes increased significantly
151	(Kruskal–Wallis chi squared = 25.02, df = 14, $P = 0.0343$) over time and reached an average of
152	65.7 g/litter box/week (or 394 g/6 litter boxes/week) in the 14th week (Fig. 6a). In contrast, the
153	weight of feces on the ground at the five popular defecation sites decreased significantly
154	(Kruskal–Wallis chi squared = 25.48, df = 14, $P = 0.0301$) over time after the litter boxes were

155	provided and reached 0 g from the 12th until the 14th week of the experiment (Fig. 6b).
156	
157	3.3. Control sites
158	The cat feces on the ground at the control sites (where no litter boxes were provided) did not
159	decrease over time. The feces in K park and U shrine remained the same, and the feces in P
160	small park increased over the 18-week period (Fig. 7). The total weight of feces over the 18
161	weeks in K park, U shrine, and P small park were 5.4, 1.8, and 3.4 kg, respectively. Although
162	observations using trail cameras were not permitted in these areas, we visually observed and
163	identified 20, 1, and 11 cats in K park, U shrine, and P small park, respectively, but we could not
164	determine which cats were responsible for the feces on the ground.
165	
166	4. Discussion
167	Cats A, B, and C were responsible for most of the feces left on the ground in the H temple
168	area. The other cats observed in the H temple grounds were either temporary visitors or
169	passersby. Before providing the litter boxes, the total weight of feces produced by the three cats
170	at the five popular defecation sites was 939 g over a 4-week period (an average of 78.3

171	g/cat/week). Seo and Tanida (2018) reported that approximately 200 free-roaming cats live in
172	the old town of Onomichi. Thus, it can be estimated that the weekly weight of feces for 200 cats
173	may reach up to 15.66 kg per week, which could have a substantial effect on the town. The
174	negative effects of free-roaming cats on wildlife species has been shown in several studies (Ash
175	and Adams, 2003; Dauphine and Cooper, 2009; Petersen et al., 2012), but the negative effects of
176	the feces of free-roaming cats has not received much attention. Stray or house cats can
177	contaminate the ground and soil with T. gondii oocysts and T. cati eggs, which are extremely
178	resistant to the environmental (Kazacos, 2001; Dabritz and Conrad, 2010; Lee et al., 2010).
179	Furthermore, the hookworms derived from domestic cats, such as Uncinaria stenocephala,
180	Ancylostoma tubaeforme, A. braziliense, and A. ceylanicum can infect humans (Bowman et al.,
181	2010; Traversa, 2012). Nagamori et al. (2018) reported that 63.9% (541/846) of the
182	free-roaming cats in north central Oklahoma, United States were infected with at least one
183	parasite and 24.9% (211/846) of the cats were infected with multiple parasites. Diakou et al.
184	(2017) showed that 24% of 150 fecal samples from the free-roaming cats living in continental
185	and insular Greece were indicative of <i>T. cati</i> infections. Nutter et al. (2004) found that the
186	percentage of feral cats seropositive with antibodies against <i>B. henselae</i> and <i>T. gondii</i> was

187 significantly higher than that of pet cats.

188	Cats A, B, and C started to use the litter boxes in the first week after placing them in the
189	temple. No special toilet training was conducted for the cats; however, cat repellent was placed
190	at the sites where the cats regularly defecated and where the temple staff did not want the cats to
191	defecate. The weekly weight of feces in the six litter boxes (g/litter box/week) increased
192	significantly (P < 0.05) and reached an average of 65.7 g/box/week (or 394 g/6 boxes/week) in
193	the final (14th) week of the study. In contrast, the weekly weight of the feces on the ground at
194	five popular defecation sites decreased significantly ($P < 0.05$) after litter box provision and
195	reached 0 g from the 12th to the final (14th) week of the study. This indicates that the provision
196	of the litter boxes had a positive effect on reducing feces in the temple grounds. However, the
197	other 14 cats observed in H temple that were either temporary visitors or passersby contributed
198	to fecal pollution elsewhere; thus, we recommend that communal cat litter boxes are provided
199	throughout the town.
200	The defecation rates of cats A, B, and C in the litter boxes were 81.3%, 88.6%, and 100%,
201	respectively, at 14 weeks, suggesting that the cats had habituated to the litter boxes. The four
202	monitored litter boxes were shared by the cats but there was a tendency for cats B and C to

203	appropriate the litter boxes for their own use, as was suggested by Neilson (2004). Olm and
204	Houpt (1988) commented that cats in multi-cat households may prefer not to defecate in the
205	same litter box as another cat or may be prevented from using the same litter box by another cat.
206	They recommended increasing the frequency of litter box cleaning to at least once a day. The
207	litter boxes in the H temple area were cleaned only once a week when the SD cards and camera
208	batteries were replaced. The cooperation of volunteer caretakers to clean the litter boxes at least
209	once a day is indispensable for the successful operation of communal litter boxes because
210	domestic cats have the ability to differentiate between feces based on fecal odors (Nakabayashi
211	et al., 2012).
212	We found that the time of litter box use varied for the three cats. This could be owing to the
213	social ranking among cats. Therefore, we recommend that more litter boxes are used than the
214	number of free-roaming cats to increase the use of litter box. Future studies should establish
215	how many litter boxes are needed and how often the boxes should be cleaned to improve the
216	litter box utilization rates.
217	The size of litter boxes also affects their utilization rates by cats. Guy et al. (2014) found

218 that cats show a definite preference for larger (86×39 cm) over regular-sized (56×38 cm)

219	litter boxes. As the boxes used in our study were repurposed plastic planters (65×25 cm)
220	similar in size to the regular-sized boxes, we recommend that larger litter boxes be tested in
221	future studies.
222	The amount of cat feces on the ground in the three control sites (where no cat litter boxes
223	were provided) did not decrease over the 18 weeks. The total amounts of feces over 18 weeks in
224	K park, U shrine, and P small park reached 5.4, 1.8, and 3.4 kg, respectively. We believe that the
225	cat feces on the ground at the three control sites would have decreased had cat litter boxes been
226	provided.
227	During the study period, we discovered cat feeders, who were either local caretakers or
228	tourists, on the temple premises and in the surrounding neighborhood. Feeding the cats
229	obviously encourages them to remain there. Feeding unowned free-roaming cats is common
230	both among households that own pets and those that do not (Natoli et al., 1999; Levy et al.,
231	2003; Finkler et al., 2011; Gunther et al., 2016; Khor et al., 2018). The presence of reliable
232	anthropogenic food sources allows a free-roaming cat colony to thrive (Tennent et al., 2009) and
233	reduces their home range size (Pillay et al., 2018). As the TNR program alone may not have a
234	great affect on urban feral cat demography, as is generally predicted (Gunther et al., 2011;

235	Gerhold and Jessup, 2018), an effective educational campaign to reduce unplanned feeding by
236	residents and tourists is necessary to control the free-roaming cat population in the old town of
237	Onomichi. We recommend promoting the provision of cat litter boxes and engaging with local
238	cat feeders to participate in the management of the litter boxes, such as cleaning and changing
239	the litter.
240	
241	5. Conclusions
242	This study shows that the provision of communal litter boxes and the application of cat
243	repellent in the territory of ownerless free-roaming cats is effective in changing their defecation
244	behavior. This will reduce the spread of zoonotic parasites by cat feces. For more effective use
245	of communal litter boxes, the optimum number of litter boxes/cat and the necessary frequency
246	of litter box cleaning should be investigated in future studies. We believe that providing
247	communal litter boxes alone will be insufficient for reducing fecal pollution by free-roaming
248	cats; thus, we propose that this be combined with an effective educational campaign directed at
249	both residents and tourists to reduce the number of free-roaming cats in Onomichi.
250	

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256	
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354	Fig. 1.	The layo	ut of the H	temple	premises	in old-to	own Onor	michi, J	apan s	howing t	he popular
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- 355 defecation sites (DS) of free-roaming cats and sites where commercial cat repellent (CR),
- 356 communal litterboxes (LB), and trail cameras (TC) were located.

358	Fig. 2. The design of the communal cat litter boxes, covered with a simple plastic roof, used in
359	the experimental study.
360	
361	Fig. 3. Changes in the number of cat defecation events in litter boxes (average number per
362	week) during the 14-week experimental period following the provision of litter boxes. A
363	Kruskal–Wallis test was used to compare the average number of defecation events between the
364	first and last week ($P = 0.09$).
365	
366	Fig. 4. Changes in the number of cat defecation events on the ground (total number per week) at
367	four popular defecation sites before and after the provision of litter boxes over an 18-week
368	experimental period. A Kruskal–Wallis test with Shirley–Williams multiple comparisons was

369	used to compare the total number of defecation events before and after the provision of
370	litterboxes.
371	* represents significance at $P < 0.01$
372	
373	Fig. 5. Changes in the a) total number of defecation events in each litter box (LB) and b) total
374	number of defecation events per hour in litter boxes by cats A, B, and C during a 14-week
375	experimental period.
376	
377	Fig. 6. Weekly changes in the a) average weight of cat feces in litter boxes and b) average
378	weight of cat feces on the ground, at five defecation sites following the provision of litter boxes
379	to free-roaming cats over a 14-week experimental period. Kruskal–Wallis tests with Shirley–
380	Williams multiple comparisons were used to compare a) the weight of feces at the start of the
381	litter box provision period (0 g) to that during the rest of the experimental period and b) the
382	weight of feces between the control (before the provision of litterboxes) and the rest of the
383	experimental period (after the provision of litterboxes). * represents significance at P \leq 0.05
384	

- **Fig. 7.** Weekly changes in the total weight (g) of cat feces on the ground at control sites over 18
- 386 weeks, in a) K park; b) U shrine; and c) P small park.

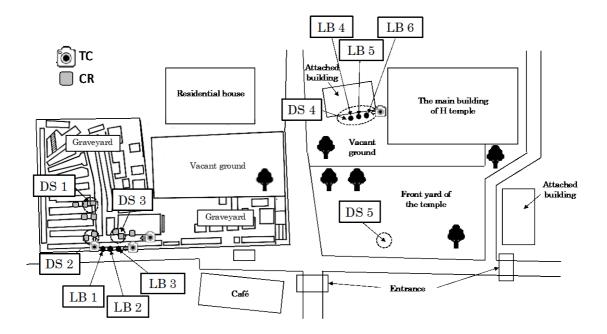


Figure 1.

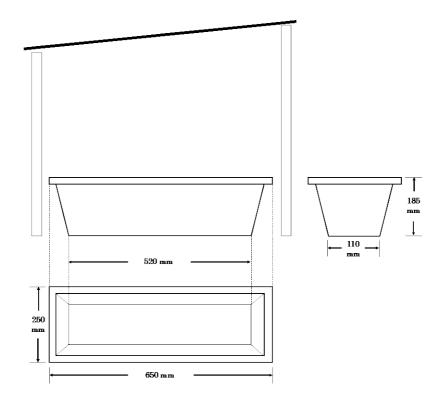


Figure 2.

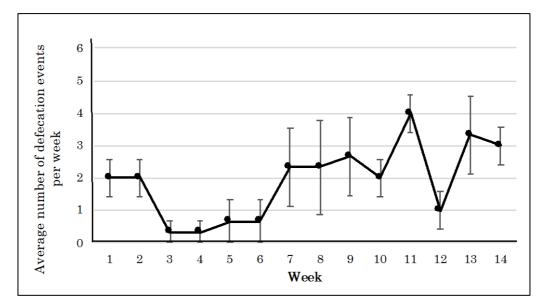


Figure 3.

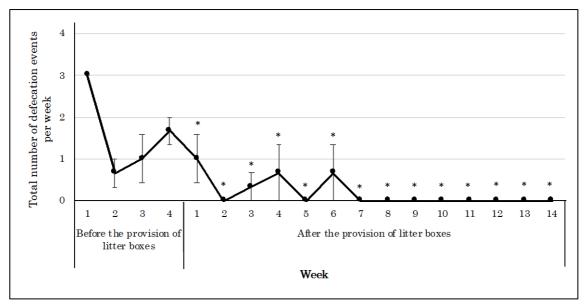


Figure 4.

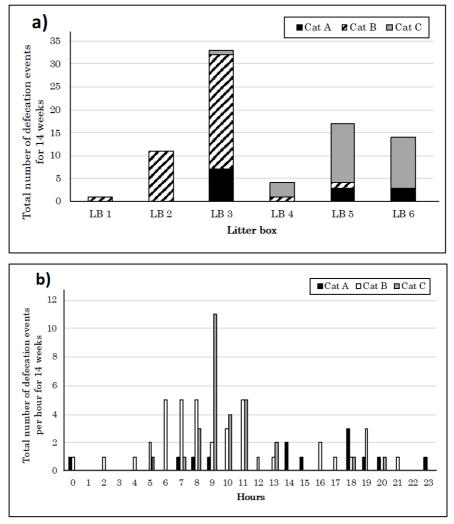


Figure 5.

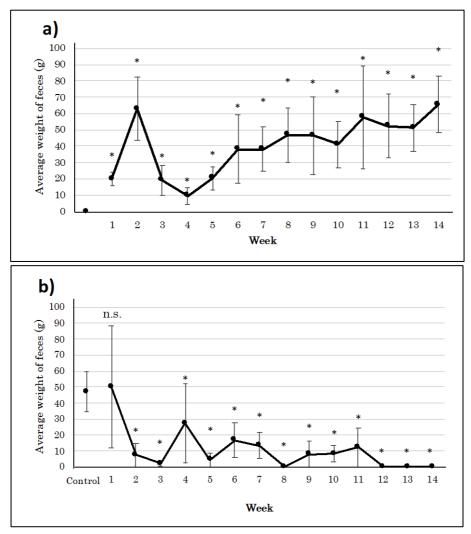


Figure 6.

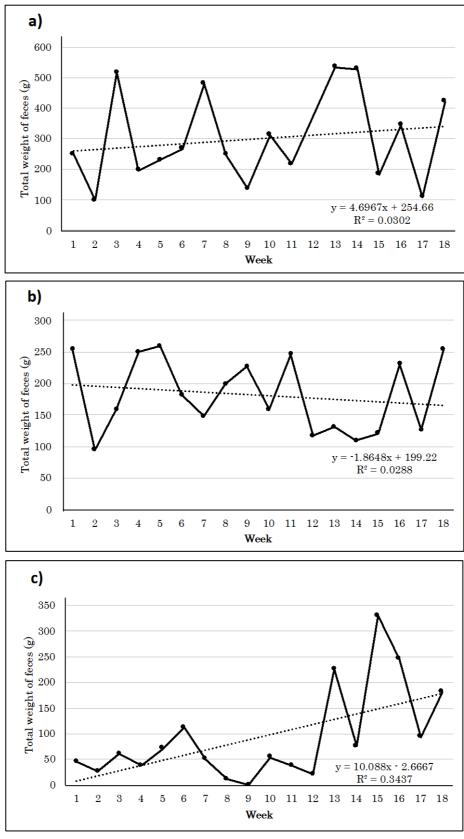


Figure 7.