

Effects of Diet Change on Shelter Dog Kennel Behaviors and Cortisol Levels

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Abstract

Ketogenic diets have shown beneficial behavior effects as well as decreases in stress in mice and humans; however, this has not been empirically evaluated in dogs. The purpose of this study was to investigate if a ketogenic diet would be effective in reducing kennel reactivity, with a focus on barking, and cortisol levels in shelter dogs that currently display kennel reactivity. We placed 33 shelter dogs on a ketogenic diet over our nine-week study period (two weeks of a gradual diet change and four weeks on a full ketogenic diet). We collected one minute video recordings for the dogs twice every collection day for a total of eight videos over our four collection periods during Weeks 0, 3, 6, and 9. Of the 18 behaviors we analyzed, six significantly differed between the baseline and treatment phases. The positive behavior change we saw was a decrease in barking during treatment compared to baseline. Despite seeing a positive effect on barking, several affiliative behaviors observed in our study showed a significant decrease from baseline to treatment (facing forward, front of kennel, gazing, and wagging tail) while facing away increased during treatment. To assess the effect of diet on cortisol levels, we collected four fecal samples starting with Week 0 and then one every three weeks after during the nine-week study period. We found a significant decrease in fecal cortisol levels between baseline and treatment phases. In summary, we saw changes in cortisol levels to indicate decreases of stress when on a ketogenic diet, as well as a decrease in our main target behavior, barking. The results indicate something as simple as a change in diet is a feasible intervention for shelters to implement in real time to address in-kennel behavior issues, such as barking.

Introduction

Being temporarily housed in an animal shelter exposes dogs to a range of potential stressors, including novelty of new stimuli, loss of attachment figures, noise exposure that can be unpredictable and intense, disruption of familiar routines like routine walks and learned feeding times, and a loss of control from the environmental contingencies (Tuber et al., 1999; Hennessy et al., 2002; Scheifele et al., 2012; Shiverdecker et al., 2013). The effects of these stimuli can be seen in both physiological and behavioral indicators of stress. Previous research found that cortisol levels in dogs increased upon entry to a shelter (Hennessy et al., 1997) and plasma cortisol levels for dogs entering a shelter were elevated by three times during their first three days in the shelter when compared to dogs living as pets in a home (Tuber et al., 1999). Behavioral impacts of shelter stressors include increased barking, destructive chewing, and stereotypic behaviors such as circling, floor licking, digging, and restlessness (Hennessy et al., 1997; Herron et al., 2014). Finding interventions to improve the welfare and behavior of sheltered dogs is critical.

Kennel reactivity, often in the form of barking at the front of the kennel, is one of the most ubiquitous and challenging behaviors, affecting not only the dogs' welfare but also the welfare of humans that spend time in the shelter environment (Protopopova, 2016). Excessive vocalization, which often is included in the barrier or kennel reactivity behavioral category, is a frequently reported behavioral issue in shelters (W. E., 2022). Excessive vocalization has been frequently reported in the scientific shelter literature as well (e.g., Tuber et al., 1999; Coppola et al., 2006; Lord et al., 2009; Kogan et al., 2012; Scheifele et al., 2012; Protopopova, 2016; Huber et al., 2017; Payne & Assemi, 2017; Jeong et al., 2020; Stephens-Lewis et al., 2022; Baldan et al., 2023). Kennel dogs' might bark due to their inability to escape from the approach of

unknown stimuli (such as dogs and people), and as such resort to barking as an alert to an intrusion of their space (Lord et al., 2009). Therefore, the simple act of living in a shelter can encourage barking amongst kennelled dogs as they are confined to close living spaces (Huber et al., 2017).

In-kennel behavior has also been linked to increased length of stays at the shelter (Protopopova et al., 2014). Though barking was not reported as increasing a dog's length of stay, dogs who moved away from the front of the kennel when approached by people had, on average, an increase in length of stay by 15 days, while dogs who paced experienced a 24-day increase and those who leaned/rubbed on the kennel walls had a median of 30 days increased (Protopopova et al., 2014) compared to dogs that did not exhibit those behaviors. Wells and Hepper (1992) found through a questionnaire that people preferred pictures of dogs who were not barking. However, Herron et al. (2014) found that dogs involved in reinforcement training for not barking were not more likely to be adopted than dogs not trained to bark less. While kennel behavior can be a deciding factor in attracting interest for adopters (Weiss et al., 2012), Protopopova et al. (2014) did not record the behaviors the adopters viewed when making their decisions to adopt or not. Moreover, Herron et al. (2014) shows how barking in particular can be less of a determining factor for adoptability, though continues to be a welfare concern for dogs (Sales et al., 1997; Coppola et al., 2006; Scheifele et al., 2012; Baldan et al., 2023).

Not only is barking a possible product of the stress-inducing nature of the shelter, but the barking produced may serve as an additional stressor for other dogs in the shelter (Schipper et al., 2008). The excessive noise can have many negative impacts on shelter dog welfare. For example, excessive barking leads to an increase in the noise levels for all the dogs living in the kennels (Coppola et al., 2006; Scheifele et al., 2012; Baldan et al., 2023). Coppola et al. (2010) found that cortisol levels within the shelter can be up to three times as high as owned dog's levels in certain circumstances; they found noise was found to be a contributing (but not the only) factor to this increase. That noise can be a stressor for dogs that can lead to behavioral responses such as decreased rest (Coppola et al., 2006). Supporting this, Gunter et al. (2019) found that when shelter dogs went on foster events, their urinary cortisol; creatinine levels significantly decreased while their longest bout of rest significantly increased.

Noise levels not only impact physiological measures of welfare in dogs, but they can also directly impact dogs' hearing, as well as that of the humans that spend time in the kennel area. There are many reports of animal shelters reaching 100dB (Sales et al., 1997; Coppola et al., 2006; Scheifele et al., 2012; Payne & Assemi, 2017; Baldan et al., 2023), a level at which the Occupational Safety and Health Administration (OSHA) indicates that exposure should be no more than 2 hours per day (U.S Department of Labor, n.d.). Coppola et al. (2006) measured the sound levels in five separate sections of the building where dogs were housed, all of which were in-door housing and ranged from 4-34 dogs in each section. Each section reached 100dB throughout the day, with the large adoptable area exceeding the 118.9dB measuring capacity of the sound reading dosimeter tool (Coppola et al., 2006). Even the smallest section housing an average of only four dogs reached 100dB throughout the day (Coppola et al., 2006). Similarly, Scheifele et al. (2012) found that noise levels over a 6-month period in the kennel environment resulted in worsening changes to the dog's hearing. Scheifele et al. (2012) compared the noise levels in the kennel environment to the standards set for human safety and found over half the dogs had reached threshold shifts indicating a change in hearing. OSHA set a 90dB limit for

human exposure to noise for safety concerns and the shelters in both studies exceeded this (U.S Department of Labor, n.d; Scheifele et al., 2012; Coppola et al., 2006). Furthermore, the World Health Organization reported that long exposure to above 50dB of noise could be harmful to mammals (Kryter, 1994).

Because of the prevalence and severity of kennel reactivity, especially excessive vocalization, interventions to decrease it are critical. Several studies have investigated potential interventions to reduce barking (Protopopova et al., 2014; Payne & Assemi, 2017; Baldan et al., 2023). Protopopova et al. (2014) recorded 289 shelter dogs to determine what behaviors they exhibited in-kennel to investigate if there is a connection between various in-kennel behaviors and length of stay in shelters. In this report, Protopopova et al. (2014) overviewed how various approaches have been made to modify shelter behavior and how barking is often chosen as the focus for the intervention like in the Luescher & Medlock (2009) and Herron et al. (2014) studies. Similarly, Payne and Assemi (2017) used respondent conditioning procedures to reduce barking. They paired a door chime to treats being delivered to the dogs by a human (Payne & Assemi, 2017). The baseline sound levels that reached up to 100dB were found to decrease after the pairing procedure was implemented (Payne & Assemi, 2017). Similarly, Baldan et al. (2023) used a positive reinforcement intervention to decrease barking in the kennels by having an experimenter walk through and stop in front of kennels to deliver treats once the barking had stopped, followed by alternative conditions to test for generalization. They found that noise levels and barking duration decreased from the beginning to the end of the experiment (Baldan et al., 2023).

Finding interventions that are feasible for shelter staff to implement that can reduce kennel reactivity and cortisol is essential. While the results of the prior interventions (Protopopova et al., 2014; Payne & Assemi, 2017; Baldan et al., 2023) are hopeful, these interventions also require additional human input to carry them out. Ensuring that interventions are easy to carry out and ideally fit into the staff or normal daily duties is recommended (Gunter & Feuerbacher, 2022). A potentially easily administrated intervention would be changing the shelter dogs' diet.

Adding supplements and altering diets has produced useful changes in dog behavior. Cannas et al. (2021) found that adding a novel nutraceutical supplement called Relaxigen Pet Dog along with tryptophan decreased stress-related behaviors, such as high alertness, tendency to hide and isolate or excessive attention seeking, and fearful of noises, people, and animals, as well as aggressive behaviors in owned dogs. Di Cerbo et al. (2017) evaluated the modulatory effects over a course of 10 days of a nutraceutical diet on behavioral changes related to chronic stress and anxiety in owned dogs, such as reactivity, excessive barking, and alertness. After the 10-day diet change, they observed increased resting by over an hour each day; along with a reduction in time spent barking from a mean of 180 seconds to 76 seconds; and decreased reactivity from a mean score of 2 (more severe) to 1 as recorded by a veterinary behaviorist (Di Cerbo et al., 2017). Hennessy et al. (2002) evaluated the effects of an experimental, higher quality diet (more animal based ingredients and proteins versus a popular commercial diet that was unnamed) versus an "industry standard" on 40 shelter dog's behavior over an 8-week period. Behaviors reduced included nondirected licking, which is linked to anxiety and stress in dogs, suggesting that the diet change may have provided a calming influence on the dogs (Hennessy et al., 2002). Additionally, a case study involving a dog that had shown increased aggression (biting and

guarding food) over a 2-year period, was reported by the owner to be more relaxed and with no signs of aggression after a diet change to a gluten-free hydrolyzed protein diet (Suñol et al., 2020). In a similar study, low protein diets combined with tryptophan supplementation reduced the mean frequency of behaviors across three behavioral categories (dominance aggression, territorial aggression, and hyperactivity; DeNapoli et al., 2000). While these studies show promising results that certain diets can produce desirable changes in the dogs' behavior, many were based on owner reports rather than direct observation of behavior (e.g., Cannas et al., 2021; DeNapoli et al., 2000), and most involved owned dogs rather than shelter dogs. Thus, our study can add to these findings by investigating how a ketogenic diet impacts shelter dog behavior.

Ketogenic diets have shown beneficial behavior effects as well as decreases in stress in mice and humans; however, this has not been empirically evaluated in dogs (Kinsman et al., 1992; Nordli et al., 2001; Pulsifer et al., 2001). Ketogenic diets are high in fat, have medium protein levels, and are low in carbohydrates. They have been shown to influence cognitive effects in humans and lab animals, especially related to epilepsy (Kinsman et al., 1992; Nordli et al., 2001; Pulsifer et al., 2001). The ketogenic diet has also shown strong neuroprotective effects in social and cognitive behaviors in humans, such as increasing social skills and interest (Kinsman et al., 1992; Nordli et al., 2001; Pulsifer et al., 2001). In a study looking into the effects of a ketogenic diet in mice, they found changes in social, meaning time spent with other mice, and repetitive behavior of autistic mice (Ruskin et al., 2017). The repetitive self-grooming behavior was significantly reduced in females after being on the ketogenic diet for 3 weeks (Ruskin et al., 2017). This study was successful in changing social and repetitive behaviors in animals and could be used as a basis for testing ketogenic diets on behaviors in other species (Ruskin et al., 2017).

The purpose of this study was to investigate if a ketogenic diet would be effective in reducing kennel reactivity, specifically barking, and cortisol levels in shelter dogs that currently displayed kennel reactivity. To assess this, we placed shelter dogs on a ketogenic diet for 6-weeks (2 weeks of a gradual diet change and 4 weeks of a full ketogenic diet); we videorecorded dogs' in-kennel behavior and collected fecal samples for cortisol analysis before and after the diet change.

Methods

Setting and Subjects

We collected data on 33 dogs at Best Friends Animal Society, Kanab, UT from August 2019 to November 2019. The study took place over nine weeks. Dogs ranged in age from 11 to 2 years (median: 5; see Table 1 for dog demographics) and were selected based on caregiver reports of showing reactive behavior (barking and/or lunging) in the kennel. None of the dogs were on a special diet at the time. Other than changing the diet and treats, all other husbandry and management of the dogs was kept the same during the study period. Dogs either lived alone, with a kennelmate, or multiple kennelmates depending on their conspecific skills. Two dogs (Dalebert and Sofia Loren) were removed from analysis later on in the study due to being adopted during our data collection phase, leaving us with a total of 31 dogs included in our analysis.

Table 1

Canine Demographics

Dog Name	Weight	Age	Sex	Reproductive Status
Timber	6.67	6y	M	N
Trinity	25.63	5y/7m	F	S
Isabel	27.67	5y	F	S
Holy Moly	33.79	5y/6m	M	N
Alice	30.5	8y/10m	F	S
Trumble	30.00	11y/1m	M	N
Ralph	29.03	9y/8m	M	N
Ricki	25.00	4y/8m	M	N
Titus	30.8	5y/6m	M	N
Akita	22.86	8y/6m	F	S
Buster	27.5	2y/4m	M	N
Bossco	25.22	2y/4m	M	N
Lana	44.82	6y/2m	F	S
Krump	32.00	7y/2m	M	N
Simon	33.61	7y/9m	M	N
Pancake	22.14	5y/2m	F	S
Ron	21.00	3y	M	N
Dalebert	27.00	10y/10m	M	N
Frankie	22.91	2y/7m	M	N
Tiponi	32.16	3y/11m	M	N
Ghostrider	31.53	4y/10m	M	N
Butch	34.88	9y/2m	M	N
Chaos	17.46	4y/11m	M	N
Sophia		11y	F	S
Loren	24.29			
Boxer	23.31	8y/7m	M	N
Little Bill	61.24	5y/9m	M	N
Castiel S	28.00	6y/10m	M	N
Gilmore	31.12	10y/10m	M	N
Peter	36.74	4y/5m	M	N
Delta	20.41	2y/6m	F	S
Barley	15.88	11y/7m	M	N
Wynonna	24.94	8y/10m	F	S
Marmalade	23.45	4y/2m	F	S

Note. Demographic data of the dogs in the study. Weight is given in kg. Age is reported in years (y) and months (m). Sex: F is female, and M is male. Reproductive status: S is spayed, and N is neutered.

Diet Change

During the first three weeks of our nine-week study, dogs were maintained on their typical diet. Starting in Week 3, dogs were gradually transitioned to a new diet consisting of Neuro Care (NC; Purina Pro Plan). The transition occurred over a two-week period: on Days 1-4 of the two-week transition, the dog's ration consisted of 25% NC and 75% their normal diet; on Days 5-9 the ration consisted of 50% NC and 50% their normal diet; finally, on Days 9-14, the dog's ration consisted of 75% NC and 25% their normal diet. After this two-week period, dogs were maintained on the NC diet for an additional four weeks. To maximize the potential behavior changes that might be produced by maintaining dogs on a ketogenic-like diet, treats for these dogs were also changed to KETO-TREATS™ (rabbit protein; Ketogenic Pet Foods, Midland, MI). If dogs were co-housed, all dogs in that run were changed to the new diet to ensure the enrolled dog was not accessing other food during the study period.

Fecal Cortisol

To assess the effect of diet on cortisol levels, we collected four fecal samples starting with Week 0 and then every three weeks after during the nine-week study period. The first two samples collected (Week 0 and Week 3) were used as a baseline during which the dogs were maintained on their normal diets. The sample for Week 6 occurred after the dogs that had been transitioned to NC had been fully on that diet for one week (plus two weeks of transition). At the time of collection for the Week 9 sample, dogs had been eating NC as their complete diet for four weeks, in addition to the two-week transition period.

Samples were collected by using a sterile, individually wrapped fecal sampling container. The lid was removed to expose an attached fecal sample stick that had a broader, spoon-like end on it. This stick was inserted straight into the fecal sample and twisted so that the spoon-like end collected a small sample from the middle of the feces, and not from the exterior which could have encountered the dirt of the kennel run.

Fecal sampling began at 7 am and continued until 5 pm, or until all samples had been collected for that day. All samples were collected either by a member of the research team or by the dog's caregivers, who had been trained by the research team on sample collection techniques. For single-housed dogs, caregivers cleaned the dog's run when they first arrived at work. If they noted a fresh sample (still had moisture on the outside of feces) in the run as they cleaned, they would take a sample. If there was no fresh sample, collectors continued to check regularly until the dog defecated again, at which point the research team member, or the caregiver collected the sample. For co-housed dogs, the caregivers cleaned the kennel and separated the dogs between the indoor and outdoor areas of the run. For the duration of the study, these dogs stayed in their separate areas, usually with the study dog being kept in the indoor portion. No samples were collected from co-housed dogs until after they were separated to ensure we could accurately ascribe the sample to the correct dog. We recorded the times when samples were collected as well. If dogs had not defecated after being fed, caregivers would sometimes take those dogs on walks to encourage defecation.

Samples were immediately placed in a cooler with ice and transferred shortly after to a freezer. They were shipped frozen overnight on dry ice back to Virginia Tech where they were kept frozen in a -80°C freezer until they were shipped to Applied Biosciences (College Station, TX) for cortisol analysis.

At each collection time point, samples were collected across two days. Samples from a given dog were scheduled to either be collected on Saturdays or Sundays. If it was not possible to obtain a sample from a Saturday dog on Saturday, collection was attempted again on Sunday. If collection on Sunday was successful for that dog, then going forward that dog would have its collection days on Sunday. However, if it was still not possible to collect a sample from a Sunday dog, that sample was missed for that collection week for the dog as the experimenters left Sunday evening to return to Virginia. Occasionally over the course of the study, a dog was adopted or had to be removed from the study for other reasons. If the dog left the study before collecting both baseline samples (Weeks 0 or 3) a new dog was added to the study to replace it. This ensured that all dogs had at least one baseline sample. Two dogs were added in this manner; Marmalade and Calvin were added in Week 3.

Behavioral Data

We collected two one-minute in-kennel behavioral videos from each dog at each of the four sample points (i.e., Week 0, 3, 6, and 9) or for three sample points for the two dogs added in Week 3. Each dog had two video recordings taken on each of the four collection days for a total of eight videos per dog. Videos were collected on the day the dog's fecal sample was collected. We recorded videos between 7-9 am prior to the dog being fed, and when caregivers reported dogs were generally more aroused. The second video was collected that same day between 10 am-12 pm, when the dogs were typically quieter in their kennels. Videos were recorded across the kennel fence with the experimenter approximately one meter from the kennel door. The experimenter wore a hat and glasses during each video recording and stared at her smartphone during the recording, rather than making eye contact with the dog. Dogs were video recorded when they were alone, so that their behavior was not impacted by that of their kennel mates. The indoor and outdoor portions of all runs could be separated by dropping a guillotine panel. Thus, for co-housed dogs, enrolled dogs were separated from kennelmates using the guillotine panel. The caregivers were also not present during our recording time.

Most videos were recorded with the experimenter in the aisle inside the kennel pod (where most visitors would approach the dog). If multiple dogs were housed in another kennel in the same pod, the experimenter would leave the pod after recording one dog, wait 30 seconds, and then re-enter the pod to record the next dog so that all dogs had the same precursor stimuli of the pod door opening and someone entering and approaching their kennel. Pair housed dogs were separated for video recording so that the behavior of one dog did not influence the behavior of the other dog. Thus, for one of a pair of cohoused dogs that were both in the study, the video recordings took place in their outside run, rather than the inside portion of the kennel. For dogs whose caregivers reported their problem behavior occurring mainly in the outside run, we also collected video recordings in the dog's outside run. For video recording dogs in the outside run, the experimenter recorded the dog while standing approximately one meter from the fencing at the end of the dog's outdoor run. For all dogs, the location where the first video was recorded of them was kept consistent throughout the study.

The same experimenter (Oberding) recorded all the videos throughout the study. While there could be habituation across sampling times, she did not interact with the dogs other than during these times. We expect with the three-week gaps and the frequent visits from other strangers during this period that the dogs would be unlikely to recognize and habituate to the experimenter from previous recording sessions.

Analysis

Fecal Cortisol

We calculated the dogs' mean fecal cortisol levels in baseline (collections 1 and 2) and the mean in treatment (collections 3 and 4) for use in our analysis. That is, if the dog had two samples in baseline, we took the mean of those two samples to create their baseline cortisol levels. If the dog only had one sample in baseline, that served as its baseline level. Because the cortisol levels were not normally distributed, we log transformed the data and conducted a paired samples t-test on the transformed data. In our graphs, we have presented the raw data for ease of visual analysis.

Behavioral Data

The in-kennel videos were coded for 28 separate behaviors (see supplementary materials for the full ethogram). The videos were analyzed by a research assistant who was trained in the behavioral definitions but was unaware of the hypothesized outcome for each behavior. Videos were scored in five-second intervals using partial interval recording, such that if a behavior occurred any time during the five second interval, the interval was scored as positive. We then calculated how many intervals each behavior occurred in as a measure of behavioral prevalence (Protopopova et al., 2014). A second independent observer coded 10% of the videos for interobserver agreement (IOA). IOA was scored by comparing interval by interval for all possible behaviors and creating a percentage agreement for each video.

We compiled the data from all four collection periods and input the data set into the programming tool RStudio. Each collection time had two videos taken, one in the morning prior to eating and one post mealtime for a total of eight videos analyzed per dog. We combined the total number of intervals of each behavior in the two videos from the same collection period to find the mean number of intervals for the entire collection for each of the four collection periods. Next, we took the average of collections 1 and 2 to create the baseline data set (pre-diet change) and averaged collections 3 and 4 for the treatment data set (post-diet change). We removed the two dogs (Dalebert and Sofia Loren) who were adopted during the study and thus did not have videos for both the baseline and treatment phases (meaning two videos for collections 1, 2, 3, and 4). A total of 31 dogs were included in the final analysis. We checked for variability of the behaviors prior to running any tests to ensure there was enough to warrant further analysis by creating a table showing the number of times each proportion of behavior showed up in the data set. Ten behaviors were excluded from analysis because five or fewer dogs displayed the behavior at any point during both the baseline and treatment phases (pawing at door, tucking tail, howling, licking kennel, chewing on kennel of bedding, rubbing on kennel wall, licking self, growling or showing teeth, scratching, and shaking off). The remaining 18 behaviors were included in our analysis (see Table 2 for those behaviors and their definitions). We checked for normal distribution and variability of the data for each behavior by making a histogram, which

showed non-normal distribution. We subsequently conducted a Shapiro-Wilk normality test on each behavior. The Shapiro-Wilk test confirmed the data were not normally distributed. Because the behavioral data were not normally distributed, we performed a Wilcoxon signed-ranked test to compare the number of intervals for which each behavior was observed during baseline and treatment.

Table 2

Behavior Definitions

Behavior	Definition	Source
Jump on cage	Both front paws make contact with the cage door that does not include lunging	(Protopopova et al., 2014)
Barking	Vocalization of very short duration and low frequency	(Protopopova et al., 2014)
Standing on cage	Both front paws make contact with the cage and dog maintains position for >1 second (ex. Dog standing to look out window)	(Protopopova et al., 2014)
Front of kennel	Located between front of kennel and up to and including the midpoint of kennel. For end kennels, this also includes interacting with the side window (looking up at the window while next to it or standing up and looking out the window).	(Protopopova et al., 2014)
Lying down	Lying down with limbs either tucked under or placed in front of body	(Protopopova et al., 2014)
Sitting	Supported by two extended front legs and two flexed back legs	(Protopopova et al., 2014)
Facing forward	Head is oriented such that an observer standing at the front of the kennel would be able to see more than the side profile of the face. For end kennels, this should be considered from the perspective of the front of the kennel or from the side window.	Adapted from (Protopopova et al., 2014)
Gazing	Eye contact with the eyes of the observer	(Protopopova et al., 2014)
Wagging tail	Tail moves perpendicularly to the dog's body	(Protopopova et al., 2014)
Moving Forward	Distance between the dog and the front of the kennel is decreased	Adapted from (Protopopova et al., 2014)
Stretching	Extending body and one or more front and/or hind-legs while remaining stationary	(Protopopova et al., 2014)
Back of kennel	Located between back wall of kennel and up to, but not including the midpoint of kennel	(Protopopova et al., 2014)
Out of sight	Not visible from the front of the cage, behavior cannot be defined	(Protopopova et al., 2014)
Facing Away	Head is oriented such an observer standing at the front of the kennel would not be able to see more than the side profile of face. For end kennels, this should be considered from the perspective of the front of the kennel or from the side window.	Adapted from (Protopopova et al., 2014)

Moving away	Distance between the dog and the front of the kennel is increased	Adapted from (Protopopova et al., 2014)
Whining	A cyclic vocalization	(Protopopova et al., 2014)
Panting	Tongue exposed with audible and/or observable breathing	(Protopopova et al., 2014)
Yawning	Opens mouth widely and inhales	(Protopopova et al., 2014)

Note. Name, definition, and source of definition for behaviors we analyzed.

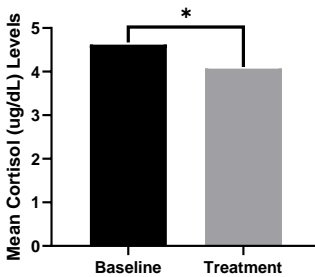
Results

Fecal Cortisol

Figure 1 shows the mean fecal cortisol level between baseline (collections 1 and 2) and treatment (collections 3 and 4). We found cortisol significantly decreased from baseline ($M = 4.83$ ug/dL, $SD = 0.05$) to treatment ($M = 4.46$ ug/dL, $SD = 0.37$).

Figure 1

Fecal Cortisol Levels



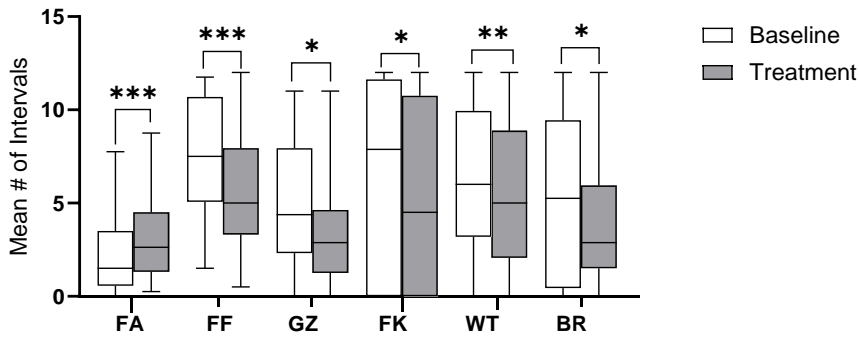
Note. Mean cortisol levels (ug/dL) of dogs measured in baseline and treatment phase. From the mean to baseline, standard deviation (SD) = 0.05. For treatment, SD = 0.37 * $p < .05$

Behavioral Data

Of the 18 behaviors we analyzed, six significantly differed between the baseline and treatment phases (Figure 2). Treatment significantly increased facing away ($V = 124.5$, $p = 0.03$), and significantly decreased facing forward ($V = 385.5$, $p < 0.001$), gazing ($V = 347.5$, $p = 0.001$), front of kennel ($V = 125.5$, $p = 0.02$), wagging tail ($V = 301$, $p = 0.007$), and barking ($V = 263$, $p < .05$). The additional 12 behaviors did not significantly differ between baseline and treatment (Figure 3). See Table 3 for a summary of the behavior changes.

Figure 2

Significant Behaviors



Note Box and whisker plot representing inter quartile range (IQR) and median with the topmost line representing Quartile 1 (Q1), middle line represents the median (Q2), and bottom line represents Q3. FA=facing away, FF=facing forward, GZ=gazing, FK=front of kennel, WT=wagging tail, BR=barking. * $p < .05$, ** $p < .01$, and *** $p < .001$.

Table 3

Analysis of 18 Behaviors

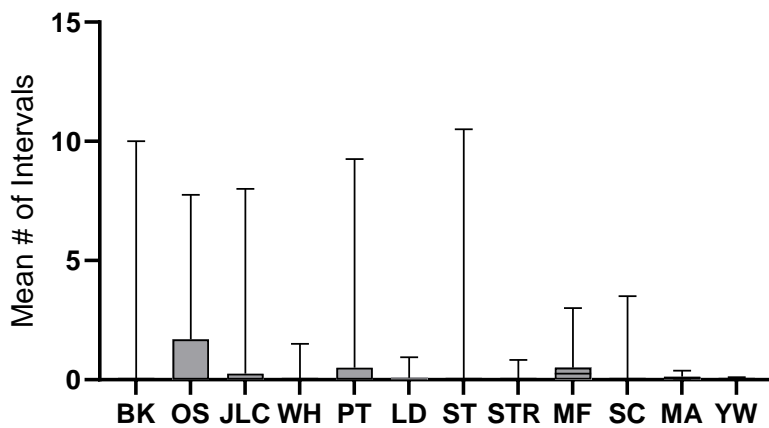
Behavior	Mean Proportions of Intervals Pre-Treatment	Mean Proportions of Intervals Post-Treatment	Direction of Change	V	<i>p</i> value	Effect Size
Back of Kennel	0.046	0.071	Increase	9.0	0.123	0.40
Barking	0.449	0.330	Decrease	263.0	0.007	0.47
Facing Away	0.210	0.276	Increase	124.5	0.027	0.41
Facing Forward	0.626	0.496	Decrease	385.5	0.002	0.56
Front of Kennel	0.533	0.457	Decrease	125.5	0.022	0.42
Gazing	0.417	0.282	Decrease	347.5	0.001	0.56
Jumping or Lunging on Cage	0.039	0.044	Increase	62.0	0.568	0.17
Lying Down	0.071	0.093	Increase	43.0	0.349	0.16
Moving Away	0.085	0.098	Increase	113.5	0.118	0.33
Moving Forward	0.030	0.043	Increase	50.0	0.124	0.27
Out of Sight	0.068	0.111	Increase	50.0	0.127	0.27
Panting	0.035	0.060	Increase	26.5	0.346	0.13

Sitting	0.085	0.027	Decrease	35.0	0.155	0.20
Standing on Cage	0.017	0.023	Increase	8.5	0.753	0.01
Stretching	0.007	0.028	Increase	15.0	0.399	0.28
Wagging Tail	0.534	0.419	Decrease	301.0	0.007	0.47
Whining	0.015	0.015	No Change	22.5	1.000	0.05
Yawning	0.009	0.003	Decrease	34.0	0.187	0.20

The collection time (baseline or treatment) was significant for back of kennel, out of sight, facing away, jumping or lunging on cage, barking, whining, panting, front of kennel, sitting, facing forward, gazing, wagging tail, moving forward, and standing on cage. Facing away significantly increased from baseline of Med = 1.50 (0.56, 3.50) to treatment Med = 2.63 (1.31, 4.50) ($V = 124.5, p = 0.03$). Facing forward significantly decreased from baseline Med = 7.50 (5.06, 10.69) to treatment Med = 5.00 (3.31, 7.94) ($V = 385.5, p < 0.001$). Gazing significantly decreased from baseline Med = 4.38 (2.31, 7.94) to treatment Med = 2.88 (1.25, 4.63). The additional 12 behaviors did not significantly differ between baseline and treatment (Figure 3).

Figure 3

Non-Significant Behaviors



Note. Box and whisker plot representing IQR and median with the topmost line representing Q1, middle line representing the median (Q2), and bottom line representing Q3. Abbreviations are as follows: BK=back of kennel, OS=out of sight, JLC=jumping or lunging on cage, WH=whining,

PT=panting, LD=lying down, ST=sitting, STR=stretching, MF=moving forward, SC=standing on cage, MA=moving away, YW=yawning.

Discussion

We evaluated the effects of a ketogenic diet on shelter dog fecal cortisol levels and in-kennel behavior. We found a significant decrease in fecal cortisol levels between baseline and treatment phases. We also found a significant decrease in barking from baseline to treatment, which is a positive outcome resulting from our intervention. However, the treatment also decreased affiliative behaviors such as facing forward, gazing, front of kennel, and wagging tail. Ketogenic diets have commonly been prescribed for dogs with idiopathic epilepsy but have also shown effects in treating ADHD-related behaviors in humans (Packer et al., 2016). The changes seen in fecal cortisol levels from baseline to treatment may signify ketogenic diets have a positive effect on in-kennel stress levels, however the majority of behavior changes did not show beneficial results. While we saw a reduction in the behavior of interest (barking) we also saw changes that might not be beneficial, such as the decrease in facing forward and front of kennel as well as an increase in averting the gaze and staying in the back of the kennel when switched to the ketogenic diet.

Our results support previous research, which suggests diet can have an impact on cortisol. As diet changes are easy to implement, and there was a useful impact on our dogs' cortisol levels and welfare, this supports our suggestion of using diet to impact shelter dog welfare. Sechi et al. (2017) tested the effects of a nutraceutical diet on stress parameters with 69 dogs evaluated at a veterinary clinic over a 45-day trial. They found that cortisol significantly decreased from their control to treatment groups following the intervention (Sechi et al., 2017). Similarly, we saw a decrease in cortisol levels upon implementation of our treatment diet. While this shows support for how diet can have an impact on cortisol levels, it should be noted that the environments of these two studies varied, with Sechi et al. (2017) occurring in a veterinary clinic with our own in a shelter and the diets given differed as well. Furthermore, a diet containing caseinate hydrolysate (an ingredient found in cow milk) was tested on a colony of 40 Beagles housed in a shelter for signs of stress (Palestrini et al., 2010). Palestrini et al. (2010) found that over a 60-day intervention period, the plasma cortisol levels significantly decreased in the dogs labeled as "anxious." As a study with a similar time period as our own, this supports our findings that diet can beneficially impact cortisol within a shelter setting. However, it should be noted that these studies utilized plasma cortisol in their analysis, whereas we used fecal cortisol. Meineri et al. (2022) looked into the effects of an added supplement of *Saccharomyces boulardii* to the diet of 25 American Staffordshire Terrier dogs housed in kennels after 35 days. They saw a significant decrease in fecal cortisol as well for the dogs in the diet supplementation group of their study (Meineri et al., 2022). Similar to the 7.7% change we saw in our fecal cortisol levels from baseline to treatment, Meineri et al. (2022) found a 6% magnitude of change in their fecal cortisol results. These results could support how diet change may be useful in reducing in-kennel cortisol for dogs.

The decrease in cortisol levels from baseline to treatment suggests our diet change could be a beneficial intervention for dogs' stress levels in the shelter. In fact, given that our study ran from August to November and the low temperature changed from approximately 11 C to 1 C, we might have expected an increase in cortisol over the same time period as dogs exposed to 4-10 C

showed increased plasma 17-hydroxycorticosteroid as compared to dogs housed in warmer temperatures of 18-20 C (Sadowski et al., 1972), a metabolite of cortisol. That our results ran counter to that suggest there could be an even larger effect than what we observed, if the temperature had been static.

The ketogenic diet also had a significant impact on in-kennel behavior in the dogs. We found a significant decrease in barking in dogs, which was one of the main behaviors we were interested in. Decreasing barking is a main goal of many shelter interventions and a variety of other interventions have shown reductions in barking from previous studies (e.g., Schipper et al., 2008; Luescher & Medlock, 2009; Herron et al., 2014; Protopopova & Wynne, 2015; Payne & Assemi, 2017; Baldan et al., 2023). Payne and Assemi (2017) used respondent conditioning procedures to reduce barking by pairing a door chime to treats being delivered to shelter dogs when the door to the kennel was opened. They saw a significant reduction in barking after their intervention. Similarly, Baldan et al. (2023) used a positive reinforcement intervention to reduce barking in a shelter by having an experimenter walk through the kennel and stop in front of each dogs' door to deliver treats once the dog was not barking. They also saw a reduction in barking after their intervention. Our intervention, however, requires the least amount of additional time as it takes place during a necessary daily task.

Despite seeing a positive effect on barking, several affiliative behaviors observed in our study showed a significant decrease from baseline to treatment. Additionally, we saw an increase in facing away, which has been associated with longer lengths of stay (Protopopova et al., 2014). Protopopova and Wynne (2015) also found that in addition to decreasing barking, their intervention using a response-independent treat delivery decreased facing away behavior in-kennel. In contrast, facing away actually increased following the diet change in the current study. The difference in results could have to do with the timing and types of our interventions, as our study replaced diet to determine in-kennel behavior while Protopopova and Wynne (2015) gave direct treatment in the moment using Pavlovian conditioning. Furthermore, we found a significant decrease in front of kennel and gazing, which differs from the nonsignificant results for these behaviors found by Herron et al. (2014). As the dogs in Herron et al. (2014) had more interaction with the experimenter during the intervention, front of kennel and gazing were uniformly high, which may be a part of why our results differed. This further supports how various interventions may be more impactful on certain behaviors than others. For wagging tail, Protopopova (2016) reports increased observations of tail wagging when the dogs were given attention by various people. The dogs in our study spent a smaller proportion of time wagging their tails in the treatment phase, which could be due to our experimenter not giving them any attention.

Furthermore, 12 of our 18 analyzed behaviors were non-significant. Back of kennel, out of sight, jumping or lunging on cage, whining, panting, lying down, sitting, stretching, moving forward, standing on cage, moving away, yawning did not occur frequently and were found to not change significantly from baseline to treatment. Of these behaviors, jumping or lunging on cage can be associated with reactivity, however it was non-significant in our study. The infrequency of occurrence and non-significance could be due to the lack of attention given by the experimenter resulting in less of a response from the dogs.

The reduction in barking has many positive impacts to shelter animal welfare. As barking can contribute to noise levels reaching up to 100dB, it is essential that we find ways to manage noise production within the shelter (Sales et al., 1997; Coppola et al., 2006; Scheifele et al., 2012; Payne & Assemi, 2017; Baldan et al., 2023). Exposure to this level of noise over six months can also cause hearing damage (Scheifele et al., 2012). Our intervention could contribute to reducing noise in shelters, which would provide a more positive environment for both the animals living there and the staff.

There were some limitations to our study. While we detected effects of the intervention on behavior, we only recorded two one-minute videos per collection of the dogs in their kennels by having an experimenter record the dogs. However, this might not be representative of the dogs' complete behavioral repertoire, especially when no human is present, or a familiar human is present. Taking longer recordings may have shown a more comprehensive account of behaviors throughout the day or using activity monitors could provide a more accurate recording of behaviors over a longer period. We also did not have any control dogs, which we could have used to better compare our results to dogs given a more standard shelter diet to see how much of an impact ketogenic diet truly had.

Conclusion

In summary, we saw changes in cortisol levels to indicate decreases of stress when on a ketogenic diet, as well as a decrease in our main targeted behavior, barking. While not all changes were in the desired direction, the impact on cortisol and barking is very hopeful, especially given that a change in diet is a feasible intervention for shelters to implement in real time to address in-kennel behavior issues and might be one facet of a strategy to improve shelter dog welfare and behavior. However, many of the affiliative behaviors observed in-kennel did not change in the expected way to support using this diet as an effective treatment for all undesirable in-kennel behaviors. Barking, however, was the main behavior of interest, and we saw a significant decrease from baseline to treatment that showed promising results for how a ketogenic diet could help modulate noise levels in shelters, which could improve the animal's welfare. Noise can have a profound effect on the welfare of the animals in a shelter and seeing both barking and fecal cortisol decrease after changing to a ketogenic diet support implementing this diet as a feasible intervention for in-kennel barking at animal shelters (Coppola et al., 2006).

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Supplemental Information

Behavior Definitions for All Behaviors Included in Beginning of Study

Behavior	Definition	Source
Front of kennel	Located between front of kennel and up to and including the midpoint of kennel. For end kennels, this also includes interacting with the side window (looking up at the window while next to it or standing up and looking out the window).	(Protopopova et al., 2014)
Back of kennel	Located between back wall of kennel and up to, but not including the midpoint of kennel	(Protopopova et al., 2014)
Lying down	Lying down with limbs either tucked under or placed in front of body	(Protopopova et al., 2014)
Out of sight	Not visible from the front of the cage, behavior cannot be defined	(Protopopova et al., 2014)
Sitting	Supported by two extended front legs and two flexed back legs	(Protopopova et al., 2014)
Pawing at door	One front paw makes contact with the door and does not stay on the door but is moved off quickly	(Protopopova et al., 2014)
Facing forward	Head is oriented such that an observer standing at the front of the kennel would be able to see more than the side profile of face. For end kennels, this should be considered from the perspective of the front of the kennel or from the side window.	(Protopopova et al., 2014)
Facing Away	Head is oriented such an observer standing at the front of the kennel would not be able to see more than the side profile of face. For end kennels, this should be considered from the perspective of the front of the kennel or from the side window.	(Protopopova et al., 2014)

Gazing	Eye contact with the eyes of the observer	(Protopopova et al., 2014)
Tucking tail	Tail held still and tightly between hind legs, may be curled under genital area or ventral side	(Protopopova et al., 2014)
Wagging tail	Tail moves perpendicularly to the dog's body	(Protopopova et al., 2014)
Moving Forward	Distance between the dog and the front of the kennel is decreased (only code during 800 videos)	(Protopopova et al., 2014)
Moving away	Distance between the dog and the front of the kennel is increased (only code during 800 videos)	(Protopopova et al., 2014)
Standing on cage	Both front paws make contact with the cage and dog maintains position for >1 second (ex. Dog standing to look out window)	(Protopopova et al., 2014)
Jump on cage	Both front paws make contact with the cage door that does not include lunging	(Protopopova et al., 2014)
Lunging	Quick diagonal forward motion; may be accompanied by barking, growling, or piloerection	(Protopopova et al., 2014)
Barking	Vocalization of very short duration and low frequency	(Protopopova et al., 2014)
Howling	Prolonged high-amplitude vocalization of varying pitch, lips drawn together while exhaling	(Protopopova et al., 2014)
Whining	A cyclic vocalization	(Protopopova et al., 2014)
Chewing on kennel	Dog repeatedly chews and bites at any of the kennel walls (>20 sec)	(Stephen, J. M., & Ledger, R. A., 2005)
Licking Kennel	Repeatedly licks, chews, and/or bites at cage door or wall	(Protopopova et al., 2014)

Rubbing on Kennel Wall	Touches kennel wall for at least 1 second while dog walks forward. Can be scored as pacing/circling if dog does this repeatedly	Our own definition
Scratching	Paw makes repeated contact with body/face; head may be angled in direction of moving limb	(Protopopova et al., 2014)
Licking Self	Oral contact with any part of body	(Protopopova et al., 2014)
Shaking off	Motions body and/or head back and forth repeatedly and rapidly	(Protopopova et al., 2014)
Yawning	Opens mouth widely and inhales	(Protopopova et al., 2014)
Stretching	Extending body and one or more front and/or hind-legs while remaining stationary	(Protopopova et al., 2014)
Panting	Tongue exposed with audible and/or observable breathing	(Protopopova et al., 2014)
Growling	Throaty, rumbling vocalization; usually low in pitch	(Protopopova et al., 2014)
