



## Springing Bottles Enrichment Toy Effect on Behaviors and Cortisol Level of Kenneled Dogs (*Canis lupus familiaris*)

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### ABSTRACT

#### Key words:

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Environmental enrichment used in captive animals with goals of increasing the activity, behavioral diversity, utilization of the environment and reducing abnormal behaviors. The present study was designed to determine the springing feeding bottles toy ameliorative effect on behaviors and cortisol level of kenneled Caucasian dogs. Sixteen healthy kenneled Caucasian shepherd dogs were utilized under four phases: 1) habituation period, 2) baseline period (pre-enrichment phase), 3) treatment period (enrichment phase)– and 4) post-exposure period (post-enrichment phase). Each phase last for 3 days during which dogs were video recorded for state and event behaviors except the first one. Hair was collected during the three phases (pre, during and post enrichment) for cortisol analysis. The provision of the feeding enrichment shorter the lying time and stereotypies frequencies, while increased the activity level. These behaviors persisted after withdrawal of enrichment with improvement in feeding time. Hair cortisol levels also was lower during and after enrichment with a marked reduction after withdrawal. Therefore, it appears that the provision of feeding enrichment toy could improve the behavioral repertoire and decrease cortisol level in kennelled dogs, and thus enhance its welfare.

## 1. INTRODUCTION

More than thousand distinct dog breeds exist today (Morris, 2008), only 20% are recognized by various national and international kennel clubs. *Canis lupus familiaris* is a subspecies that exhibits variation in morphological and behavioral phenotypes. The domestic dog and its breeds that make up this subspecies is becoming an increasingly popular subject for behavioral research (Mehrkam and Wynne, 2014). Dog breeds are groups of individuals that strongly resemble one another based on a series of characteristics that are identifiably different from other groups (Brewer et al., 2002). However, the most accurate way to define a breed on base of behavior is still debated.

The welfare of captive dogs is considered to be severely compromised as many governmental or charity-run kennels had been occupied by many dog's despite of their value and popularity as working, sport and pets (Clark et al., 2012; Stavisky

et al., 2012; New et al., 2014). Canine behaviors are critical indices for proper housing and management; moreover, it plays a major role in determining an animal's adaptability and welfare. In captivity, feeding regimes do not fulfil their specific needs which restricted specific behaviors, so they become inactive or display abnormal behaviors (Kistler et al., 2009).

Laboratory housing systems also lack sufficient stimuli, because of limited space, so animals can't display their specific behavioral repertoire, in addition municipal shelters that rely on single-dog housing may also be of restricted size (Meghan et al., 2014). These housing conditions with restricted space allowances can lead to fear, aggression, increased self-grooming, vocalizations, repeated movement behavior (i.e. stereotypies) and coprophagy (Hetts et al., 1992; Hubrecht et al., 1992; Beerda et al., 1999a).

Stereotypies are considered abnormal or undesirable behaviors that are repeated for no apparent function

(Mason, 1991), that occur due to exposure to an ecological problem that cannot be solved by the animal, for example, pacing in fields and feather plucking in birds. These behavioral repertoires can be used as indicators of stress and may therefore indicate a compromised welfare state (Broom, 1991; Broom and Johnson, 1993; Beerda et al., 1999). Thus, monitoring and evaluating changes in animal housing should be considered (Baumans, 2005), and used as a tool to judge the adaptability of animal with its environment.

Inferences from behavioral measures can be used more easier to assess welfare (Boissy et al., 2007) in contrast to physiological ones, as relatively non-invasive, inexpensive and easily applicable for understaffed facilities (Burgdorf and Panksepp, 2006). For example, animal care staff can assess the managerial practices effect on the animal physical or social environment through time budget behaviors which indicate how an animal utilizes its time (Kagan and Veasey, 2010). From these activity budget, behavioral diversity could serve as an indicator of behavioral opportunities and degree of animals control over their environment. Captive animals showed less behavioral diversity than wild animals (Shepherdson, 2010). Recently, behavioral diversity in kenneled dogs has been increased with improving quality of life (Kiddie, 2012), and novelty (Part et al., 2014) as stated by Hirt and Wechsler (1994) that behavioral diversity can be increased in captivity through improving housing.

In the last centuries, a new husbandry management was applied for captive animals, which is an environmental enrichment (Mellen and Sevenich MacPhee, 2001). It helps in reduction of abnormal behaviors and allows animal to perform its own specific behaviors, thus, keeping the animal occupied through motivation with environmental stimulation (Honest and Marin, 2006; Shyne, 2006). These beneficial effects could result in improvement in the welfare of captive animals through increased activity, stimulation of more diverse range of behaviors, increased exploration and use of environment (Price, 2010).

A variety of animate and inanimate enrichment objects was suggested for kennelled dogs to prevent undesirable behaviors (reviewed by: Hubrecht, 1993; Wells, 2004). Pullen et al. (2010) deducted that chewable toys provided on the floor were preferred by dogs than hanged toys. Inanimate enrichment in the form of food-filled toys was found to promote desirable behaviors in shelter dogs (Schipper et al., 2008). The provision of such toys seemed to enhance adaptability and public preference for shelter dogs (Wells and Hepper, 1992; Luescher and Medlock,

2009), because locking for long times in shelters has been correlated with a worsening of behavior and poor welfare (Wells et al., 2002).

Dishman et al. (2009) found that in ring tailed lemurs (*Lemur catta*), feeding enrichment increased activity levels and reduced undesirable behaviors. An increase in exploratory behavior was also reported by providing captive Maned Wolves (*Chrsocyon brachyurus*) with hidden food (Cummings et al., 2007). Moreover, the time spent feeding increased with artificial feeding devices presented to red foxes (*Vulpes vulpes*), which appeared to stimulate food searching behavior (Kistler et al., 2009). Kruger (1996) indicated that natural foraging and feeding behaviors in opportunistic carnivores, such as the African wild dog can be stimulated through the use of feeding enrichment.

Environmental enrichment produce its beneficial effects through two clear effects: first, brain structural changes that could improve cognitive performance (Wolfer et al., 2004) and second, it help the animal to display its ethological requirements (Newberry, 1995). This increase in cognitive ability and display of behavioral diversity specific for species, could improve the adaptability of the animal on the long run towards the environment (Larsson et al., 2002; Abou-Ismaïl, 2011; Abou-Ismaïl and Mandl, 2016). The latter is also rewarding for the animal (Spruijt et al., 2001) and may ameliorate the behavioral alteration induced by stress (Vander Harst et al., 2005).

Hair cortisol level could be used as a reliable measure for stress and welfare in dogs and other mammals. In contrast to blood and salivary cortisol which reflect instant cortisol secretion, the cortisol levels in hair samples provide a retrospective measurement over a longer period of time (Accorsi et al., 2008; Ouschan et al., 2013).

Environmental enrichments can improve animal life quality; however, these techniques are most commonly used in neuroscience studies (Fischer et al., 2016). Although, it was reported that environmental enrichment could be efficient in promoting welfare in dogs kept in laboratories through reducing serum cortisol concentrations and anxiety behaviors, while increasing desirable behaviors. This reduction in blood cortisol levels was repeatable and associated with positive behavioral changes, although, the effect was short in duration and appeared to vary with the breed of dogs (Willen et al., 2017). Thus, the accumulation of cortisol in hair may be a useful method to evaluate stress and welfare state of dogs.

The information available on the impact of feeding enrichment on behaviors of kenneled dogs is limited.

Thus, the objective of the present study is to determine the ameliorative effect of the springing bottles toy as a feeding enrichment on behaviors and cortisol level in hair of kenneled Caucasian dogs. The hypothesis of this study is that the provision of feeding enrichment will improve the welfare of kenneled dogs through increasing both feeding behavior and activity levels while, reducing stereotypies, coprophagy and hair cortisol.

## 2. MATERIALS AND METHODS

### 2.1. Ethical statement:

Experimental procedures were approved from Alexandria and Damanhur University Animal Ethics Committee. This study was conducted without subjecting animals to any degree of suffering or stress to preserve and safeguard their welfare.

### 2.2. Subjects, housing and daily care:

This experiment was conducted at Al-max Veterinary hospital, Alexandria, Egypt. Sixteen healthy kenneled Caucasian shepherd dogs (*Canis lupus familiaris*) were utilized in this study. The dogs were housed individually in metal cages. Conspecifics couldn't observe each other, although they still had the ability to smell and hear each other. The enclosures were cleaned daily. Water was available *ad libitum* in plastic bowls and the animals were fed once a day at 9.00 am daily. The daily care procedures were sustained along the study according to normal practices included in the routine handling of animal by the caretaker.

### 2.3. Experimental procedures:

The experimental procedures consisted of four phases according to recommendation of Schipper et al. (2008) as follows: 1) Habituation period for the presence of cameras and researchers. 2) Baseline period (pre-enrichment phase), in which the subject animals were observed before the provision of enrichment. 3) Treatment period (enrichment phase), feeding enrichment items (springing bottles toy) were provided inside the kennel after feeding. 4) Post-exposure period (post-enrichment phase), enrichment items were removed. Each phase last for 3 days during which dogs were video recorded for state and event behaviors except the first one. Behaviors of dogs were video recorded for 20 min/session for four hours/day and total of 12 sessions/experimental period between 9.30 am and 13.30 pm.

### 2.4. Behavioral data collection and video analysis:

Video recordings were analysed for state and event behaviors (see Table-1: Behavioral Ethogram) by two observers blind to the procedures. Behaviors were recorded using continuous focal sampling method.

### 2.5. Collection of hair samples:

Approximately 100-150 mg of ischiatic hair was collected, apart from the skin by 5 mm, from each dog using grooming clipper of pets. Hair sample of individual dog was collected into a plastic bag and stored at room temperature until analysis.

### 2.6. Extraction of cortisol:

Hair sample was washed in 5 ml of isopropanol twice through gentle rotation for about 3 min then dried for 3 days at room temperature and fragmented into fragments, 1-3 mm in length then placed inside a glass vial. Absolute methanol (2 ml) was added to the glass vial containing the hair fragments. A control sample was made of 2 ml aliquot of methanol without hair. The vials were agitated gently at room temperature for 24 h then centrifugated at 1500 rpm for 5 min. Supernatant was decanted, then, evaporated the organic solvent at 60°C for 3 h. About 250 µl of cortisol assay kit buffer were used to re-dissolve extracts.

Cortisol level was estimated using ELISA kit (DRG Diagnostics, Marburg, Germany) according to the method described by Bennett and Hayssen (2010). According to the manufacturer's guideline, the minimum cortisol concentration detectable was 0.003 µl /dL, while the mean intra- and inter-variation were 9.52% and 14.75%, respectively. The intra- and inter- CVs were calculated depending on the cortisol concentrations of the kit standards.

### 2.7. Statistical analysis:

Data of behavior were analysed using two-way analysis of variance (ANOVA) in SAS software (version 6, 4<sup>th</sup> Edition, SAS Institute, Cary, NC, USA.). Means ± SEM were used to express the data, and p values < 0.05 were considered significant in all the tests, unless mentioned otherwise. Analysis of the significant main effects of the experimental treatment was performed using a Duncan's multiple range test. Data of hair cortisol level were analyzed with One-way ANOVA, Newman-Keuls multiple comparison tests with GraphPad Prism 5 (San Diego, CA, USA). All declarations of significance depended on P values < 0.05.

## 3. RESULTS

### 3.1. Effect of springing bottles feeding enrichment toy on behaviors of Kenneled dogs:

As overall view, pre, during and post feeding toy enrichment effect on behaviors were shown in Table (2). Lying time decreased during enrichment period compared to that of pre- and post-enrichment. The toy interaction frequency and time indicated a pronounced effect during its presence. There was a gradual increase in walking activity throughout the study phases. The bow play increased through

enrichment than during pre and post enrichment phases. Hind leg behavior and exploration increased during and post-enrichment period than pre-enrichment. During post-enrichment period grooming and time spent feeding increased than through other periods. The stereotypes exhibited a progressive diminish throughout the study phases. Regarding the interaction of enrichment phases with days per phase effect (Table 3), the longest standing time was at day 1 pre-enrichment, and the shortest at day 2 during enrichment. Lying time was longest at day 2 pre-enrichment, and shortest at day 1 during enrichment. The toy interaction frequency was increasing reaching its highest value at day 3, with no significant difference for time spent interacting. The hind leg and bow play position showed the highest frequency at day 3 during enrichment and the lowest at day 1 pre-enrichment and day 1 and 3 post-enrichment, respectively. However, walking showed the highest value at day 1 and 2 post-enrichment and

exploration at day 2 post-enrichment while, the lowest at day 2 and 3 pre-enrichment and day 2 pre-enrichment respectively. The grooming frequency was at its highest value at day 1 post-enrichment and lowest at day 1 and 3 pre-enrichment.

Stereotypes displayed its highest mean value at day 3 pre-enrichment and the lowest at day 2 during enrichment and diminished at the post-enrichment days. The longest feeding time and highest drinking frequency were reported significantly from day 3 during enrichment to day 3 for feeding and day 2 post-enrichment for drinking respectively.

**3.2. Effect of springing bottles feeding enrichment toy on hair cortisol level of Kennel dogs:**

Cortisol levels revealed significant differences during, pre and post enrichment (Fig. 1). Dogs had higher cortisol values during the pre-enrichment period than post enrichment time (P= 0.009), with a marked reduction during the post enrichment period in comparison to enrichment period (P= 0.0095).

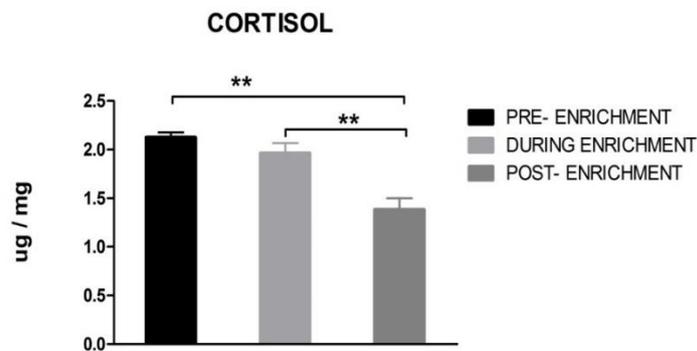
**Table 1. Behavioral Ethogram for state and event behaviors.**

Item	Description
<b>Resting</b>	
Standing	Stand in a position with three or four limbs on ground
Sitting	Sit with hind-limbs flexed and fore-limbs extended
Lying	Lying on lateral or ventral recumbency
<b>Movement activities</b>	
Walking	Moving inside the enclosure at any speed
Hind leg position	Standing with back legs upward and fore-legs free or supported against object
Exploration	Dog is exploring its surroundings (e.g. sniffing, licking and scratching)
Bow play position	The anterior part of body is lowered appearing to be sitting on its fore legs with the posterior part raised indicating a playful intention
<b>Body care</b>	
Grooming	The animal either scratches, bites or licks its own body while being sitting/standing/lying
Shaking	Shaking of the body/head
<b>Abnormal behaviors</b>	
Stereotypies	A complete turn in circling, or route in pacing
Coprophagia	Eating their own faeces
Panting	Deep and quick breathing with mouth open
<b>Elimination</b>	
Urination/defecation	The animal either raises its hind-leg or being in a squatting position
<b>Ingestive</b>	
Feeding	Eats food with its mouth in the plate
Drinking	Dog drinks with its mouth in the water bowl
<b>Toy interaction</b>	
Toy interaction frequency	Number of times dog chewing, licking and carrying the toy
Toy interaction time	Time spent by a dog in exploration of toy (e.g. chewing, licking, carrying, etc.)

**Table 2. Effect of springing bottles enrichment toy on behaviors of Kennelled dogs during different phases.**

Behaviors	Pre-enrichment	During enrichment	Post-enrichment
<b>Resting</b>			
Standing	5.86±0.70 <sup>a</sup>	4.69±0.67 <sup>a</sup>	6.34±0.66 <sup>a</sup>
Sitting	3.49±0.62 <sup>a</sup>	2.34±0.40 <sup>a</sup>	3.38±0.51 <sup>a</sup>
Lying	9.15±0.92 <sup>a</sup>	6.36±1.05 <sup>b</sup>	7.46±0.87 <sup>ab</sup>
<b>Movement activities</b>			
Walking	1.28±0.34 <sup>c</sup>	7.28±1.41 <sup>b</sup>	16.39±1.96 <sup>a</sup>
Hind leg	0.56±0.21 <sup>b</sup>	1.78±0.66 <sup>ab</sup>	2.25±0.57 <sup>a</sup>
Exploration	0.78±0.20 <sup>b</sup>	2.53±0.47 <sup>a</sup>	2.56±0.79 <sup>a</sup>
Bow play	0.31±0.09 <sup>ab</sup>	0.72±0.27 <sup>a</sup>	0.17±0.09 <sup>b</sup>
<b>Body care</b>			
Grooming	0.47±0.18 <sup>b</sup>	0.81±0.19 <sup>b</sup>	2.92±0.47 <sup>a</sup>
Shaking	0.03±0.03 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.08±0.06 <sup>a</sup>
<b>Abnormal behaviours</b>			
Stereotype	4.67±1.02 <sup>a</sup>	2.03±0.76 <sup>b</sup>	0.00±0.00 <sup>c</sup>
Coprophagia	0.03±0.03 <sup>a</sup>	0.08±0.06 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Panting	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
<b>Elimination</b>			
Urination	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.06±0.04 <sup>a</sup>
Defecation	0.06±0.04 <sup>a</sup>	0.08±0.05 <sup>a</sup>	0.25±0.13 <sup>a</sup>
<b>Ingestive</b>			
Feeding	0.33±0.16 <sup>b</sup>	0.24±0.12 <sup>b</sup>	0.85±0.22 <sup>a</sup>
Drinking	0.36±0.14 <sup>a</sup>	0.50±0.18 <sup>a</sup>	0.81±0.16 <sup>a</sup>
<b>Toy interaction</b>			
Toy interaction frequency	0.00±0.00 <sup>b</sup>	20.19±3.65 <sup>a</sup>	0.00±0.00 <sup>b</sup>
Toy interaction time	0.00±0.00 <sup>b</sup>	4.19±0.71 <sup>a</sup>	0.00±0.00 <sup>b</sup>

Means within the same row carrying different superscript letters are significantly different at  $P \leq 0.05$ .



**Fig. 1.** Springing bottles feeding enrichment toy effect on hair cortisol level before, during and after exposure to enrichment in kennelled dogs, \*\* ( $p < 0.01$ ) is significant.

#### 4. DISCUSSION

The kennel environment, even for short periods, is a psychogenic stressor for most dogs due to its novel surroundings and the separation from social conspecifics. Rescue shelters also especially those under-resourced may lead to poor welfare (Kiddie et al., 2017). To improve the welfare of kennelled dogs, a problem-solving game could be used, as it improves an individual's physical and cognitive capabilities, and therefore its welfare (Zilocchi et al., 2018). This study was designed to investigate the effect of locally manufactured cheap accessible object added to cages

of dogs on their behaviors to determine whether it is possible to be used by shelter organisations with minimal pressure on time and financial budgets - for improving their dogs welfare. The hypothesis was that the provision of feeding enrichment toy would increase both feeding behavior and activity levels while, reduce stereotypes and hair cortisol level. As hypothesized, the main behavioral results obtained can be summed as follows: lying time decreased, activity levels - represented in walking, bow play, hind leg position and exploration- increased, while stereotypes decreased during the treatment phase.

**Table 3. Effect of interaction between springing bottles enrichment toy phases and days within each phase on behaviors of Kennel dogs**

Behaviors	Pre-enrichment			During enrichment			Post-enrichment		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
<b>Resting</b>									
Standing	7.92±1.37 <sup>a</sup>	3.79±0.75 <sup>bc</sup>	5.88±1.2 <sup>abc</sup>	6.85±1.50 <sup>ab</sup>	2.60±0.71 <sup>c</sup>	4.61±0.87 <sup>abc</sup>	7.03±1.34 <sup>ab</sup>	6.55±0.89 <sup>ab</sup>	5.43±1.22 <sup>abc</sup>
Sitting	4.38±1.30 <sup>a</sup>	2.58±0.84 <sup>a</sup>	3.50±1.04 <sup>a</sup>	2.38±0.79 <sup>a</sup>	2.32±0.69 <sup>a</sup>	2.33±0.67 <sup>a</sup>	3.90±0.81 <sup>a</sup>	3.32±0.85 <sup>a</sup>	2.91±1.02 <sup>a</sup>
Lying	6.67±1.32 <sup>bcd</sup>	11.75±1.29 <sup>a</sup>	9.04±1.85 <sup>abc</sup>	3.53±1.38 <sup>d</sup>	10.38±1.99 <sup>ab</sup>	5.15±1.52 <sup>cd</sup>	6.42±1.26 <sup>bcd</sup>	6.30±1.70 <sup>bcd</sup>	9.66±1.47 <sup>abc</sup>
<b>Movement activities</b>									
Walking	2.42±0.88 <sup>d</sup>	0.50±0.19 <sup>d</sup>	0.92±0.29 <sup>d</sup>	2.92±1.30 <sup>d</sup>	4.75±1.95 <sup>cd</sup>	14.17±2.61 <sup>ab</sup>	19.92±2.91 <sup>a</sup>	19.33±4.23 <sup>a</sup>	9.92±2.14 <sup>bc</sup>
Hind leg	0.25±0.13 <sup>b</sup>	0.42±0.34 <sup>ab</sup>	1.00±0.52 <sup>ab</sup>	1.33±0.48 <sup>ab</sup>	0.75±0.43 <sup>ab</sup>	3.25±1.86 <sup>a</sup>	2.17±0.91 <sup>ab</sup>	3.17±1.39 <sup>ab</sup>	1.42±0.42 <sup>ab</sup>
Exploration	0.92±0.42 <sup>b</sup>	0.17±0.11 <sup>b</sup>	1.25±0.39 <sup>b</sup>	2.50±0.92 <sup>ab</sup>	2.25±0.68 <sup>ab</sup>	2.83±0.91 <sup>ab</sup>	0.67±0.43 <sup>b</sup>	4.92±2.12 <sup>a</sup>	2.08±0.58 <sup>ab</sup>
Bow play	0.42±0.19 <sup>abc</sup>	0.33±0.14 <sup>abc</sup>	0.17±0.11 <sup>abc</sup>	0.08±0.08 <sup>bc</sup>	1.00±0.54 <sup>ab</sup>	1.08±0.61 <sup>a</sup>	0.00±0.00 <sup>c</sup>	0.50±0.26 <sup>abc</sup>	0.00±0.00 <sup>c</sup>
<b>Body care</b>									
Grooming	0.17±0.11 <sup>d</sup>	1.08±0.47 <sup>cd</sup>	0.17±0.17 <sup>d</sup>	0.67±0.36 <sup>cd</sup>	0.67±0.26 <sup>cd</sup>	1.08±0.40 <sup>cd</sup>	3.83±1.07 <sup>a</sup>	2.00±0.72 <sup>bc</sup>	2.92±0.57 <sup>ab</sup>
Shaking	0.08±0.08 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.08±0.08 <sup>a</sup>	0.17±0.17 <sup>a</sup>	0.00±0.00 <sup>a</sup>
<b>Abnormal behaviors</b>									
Stereotypy	1.17±0.21 <sup>bc</sup>	4.17±1.81 <sup>b</sup>	8.67±2.02 <sup>a</sup>	3.42±0.88 <sup>bc</sup>	0.58±0.29 <sup>bc</sup>	2.08±2.08 <sup>bc</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>
Coprophagia	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.08±0.08 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.17±0.17 <sup>a</sup>	0.08±0.08 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Panting	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
<b>Elimination</b>									
Urination	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.17±0.11 <sup>a</sup>	0.00±0.00 <sup>b</sup>
Defecation	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.17±0.11 <sup>ab</sup>	0.17±0.11 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	0.08±0.08 <sup>ab</sup>	0.17±0.17 <sup>ab</sup>	0.50±0.34 <sup>a</sup>	0.08±0.08 <sup>ab</sup>
<b>Ingestive</b>									
Feeding	0.00±0.00 <sup>b</sup>	1.00±0.43 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.72±0.31 <sup>ab</sup>	0.67±0.29 <sup>ab</sup>	0.88±0.41 <sup>ab</sup>	1.00±0.43 <sup>a</sup>
Drinking	0.25±0.18 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	0.83±0.34 <sup>ab</sup>	0.33±0.22 <sup>ab</sup>	0.42±0.34 <sup>ab</sup>	0.75±0.37 <sup>ab</sup>	0.67±0.36 <sup>ab</sup>	1.08±0.26 <sup>a</sup>	0.67±0.22 <sup>ab</sup>
<b>Toy interaction</b>									
Toy interaction frequency	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	18.42±6.5 <sup>ab</sup>	13.83±5.53 <sup>b</sup>	28.33±6.67 <sup>a</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>
Toy interaction time	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	5.27±1.39 <sup>a</sup>	3.33±1.22 <sup>a</sup>	3.96±1.08 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>

Means within the same row carrying different superscript letters are significantly different at  $P \leq 0.05$ .

Most recorded behavioral changes continued post-enrichment as walking, hind leg position and exploration, in addition to improvement in feeding time and diminish of stereotypies. The hair cortisol value decreased during and post enrichment periods with a marked reduction post enrichment.

The decrement in lying time reported here is supported by the study of Kiddie et al. (2017) who reported that treatment with coconuts, cardboard bed or cardboard partition decreased yawning frequencies, lying and sitting down time without difference between them, which could indicate the active engagement of dogs with the enrichment objects, so support the hypothesis stating that the used objects were enriching. The grooming frequency decrease through the enrichment phase may be attributed to this behavior association with chronic stress in dogs and so it increases post-enrichment (Beerda et al., 2000) as a displacement activity in contexts of conflict (Hennessy et al., 2006). Previous studies on ACTH-induced behavioral syndromes, suggested, inclusion of displacement behaviors as grooming and yawning (Bertolini, et al., 1998). This adds a further support that the treatment was enriching. In contrast, grooming had been found to be associated with

positive welfare state (Boissy et al., 2007), therefore these behaviors may be influenced more by individuality than by treatment.

In the current study, stereotypes revealed a progressive significant diminish with a gradual increase in walking frequency, which indicate the enriching effect of these objects. Previous studies suggested that singly housed dogs may be predisposed to stereotypic behaviors, such as circling, digging, floor licking, and frequent changes from one state of locomotion to another (i.e. changing from walking to jumping, from standing to spinning, and from sitting to pacing) (Hubrecht et al., 1992; Beerda et al., 1999a). Such behaviors have been correlated with chronic stress and may be indicators of poor welfare (Beerda et al., 1997; Beerda et al., 1999 a, b). There was a trend toward decreasing stereotyping (i.e. repetitive behaviors) during the period of enrichment, and when enrichment was discontinued, and an increase in the activity level (Mason and Latham, 2004). Thus, interaction with toys may decrease a dog's response to environmental factors (e.g. noise, strange people or dogs), arousal and its related behaviors, such as excessive barking, digging, trying to escape and destroying property (Mason et al., 2007). In which, it stimulates the

animal physical activity and behavioral diversity, in addition to helping the animal to obtain food (Clarke et al., 2005). As reported by Dishman et al. (2009), the activity level increased and abnormal behaviors decreased in captive ring-tailed lemurs (*Lemur catta*) treated with feeding enrichment.

Skibieli et al. (2007) showed that the provision of food enrichment increased active behaviors of captive felids. Parker et al. (2006) revealed that the enrichment in captive Vicugna (*Vicugna vicugna*) reduced stereotypic behaviors like pacing around and head-shakings. Dogs that had mostly used the enrichment device was showing a high cortisol levels, followed by a decrease when it was re-introduced, indicating that feeding enrichment device was valued to the dogs, and the welfare of some individuals had been enhanced during enrichment provision (Mason and Latham, 2004). Furthermore, Price (2010) deduced that enrichment stimulated searching for food which could explain the gradual increase in the movement activity from pre- to enrichment time and sustained post-enrichment in the current study.

The increased frequency of exploration during and post-enrichment period in this study could be supported by the findings of previous studies. Wells and Egli (2004) reported that activity levels and exploration increased, while, undesirable behaviors reduced due to enrichment in zoo-housed black-footed cats' (*Felis nigripes*) that could be advantageous for improving animal well-being. Cummings et al. (2007) showed that the activity and exploratory behaviors increased significantly also in captive Maned Wolves (*Chrysocyon brachyurus*) by providing hidden food Ings et al. (1997), also reported that the specially constructed wood piles used for hiding food increased the searching behavior around the enclosure in Bush dogs (*Speothos venaticus*). Mixing four intact food pellets, and other four pellets that had been broken into numerous small pieces with the bedding material at the time of cage cleaning as food enrichment for group-housed rats increased -among others- exploration and interaction with enrichment, and improved welfare (Abou-Ismael et al., 2010). More importantly, they attributed these behavioral changes were due to the presence of the enrichments themselves in the cages (indirect effects) and not merely due to interaction of rats with the enrichment items itself.

This increased level of exploration reported in the current work could be attributed to the fact that problem-solving tasks could evoke an immediate positive emotional state in animals, as a means to motivate dogs to explore and solve problems, even if the true benefit of the behavior is on the long term

(McGowan et al., 2014; Zilocchi et al., 2018). Supporting the idea that opportunities to solve problems, making decisions and exercise cognitive skills are important to an animal's emotional experiences and ultimately its welfare as positive affective feelings. This may help animals to better identify behaviors that are biologically useful and to encourage them to carry out these behaviors to their benefit on the long term.

Post-enrichment increased feeding time may be due to that feeding enrichment encourages animals to perform natural foraging and feeding behaviors with the use of food as a reward (Young, 1997). In addition, it helps animal also to display more behavioral repertoire on the short run, therefore improving the welfare of kennelled dogs (Schipper et al., 2008). Increased foraging behavior may decrease unwanted behaviors, and also helps to increase physical activity, which can benefit the animal's physical condition. The more pronounced interaction time with the toy during the enrichment phase, could be supported by Hubrecht, (1993) who reported that about a quarter of time was spent exploring toy immediately after introduction to kennelled dogs. Similar findings were also reported in zoo-housed canids (Ings et al., 1997) and other species (Shepherdson et al., 1993).

Dogs that were captured and housed in kennels became more stressed as the kennel environment presents novelty and a mixture of different stressors, such as unusual experience and unfamiliar environment. It is well known that dogs show glucocorticoids elevation when exposed to novelty (Tuber et al., 1999).

Part et al. (2014) observed that cortisol levels were higher in domestic dogs housed in the kennel than those in-home environments. In this study cortisol concentration decreased during and post enrichment indicating that capture in general represents a stressful situation for dogs. This reduction in cortisol may be attributed to the fact that it is associated with chronic stress in dogs, so the decrease reported post-enrichment. Similarly finding was observed by Willen et al. (2017) who reported reduction in cortisol concentration through environmental and/or social enrichment in dogs. In the current study, the reduction in cortisol concentration corroborates well with the behavioral display indicating active engagement of dogs with the enrichment object, supporting the hypothesis that the object presented was enriching and efficient in promoting welfare in dogs.

## 5. CONCLUSION

Feeding enrichment toy had a positive impact on dogs' behavior, through increasing activity levels and reducing stereotypies, and decreasing cortisol level. Therefore, feeding enrichment toys could be applied as a cheap and sustainable enrichment to dogs living in highly constrained environments.

### 6. Limitation of the study

A larger sample size could be utilized with addition of a control group for comparison. Moreover, using more behavioral observational methods in further studies appear important for detecting less observed behaviors as well. Future studies should also consider long-term effects under social housing conditions.

### 7. DISCLOSURE OF INTEREST

All authors had no conflict of interest.

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