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Impact of Short-Term Aromatherapy and COVID-19 Pandemic on Stereotypic Behavior and Cortisol Concentration in Captive Big Cats

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Zoological garden, Lions, Tigers, ELISA, Cortisol level, Captive behavior

ABSTRACT

This study examines the impact of sensory enrichment on the well-being of captive lions *(Panthera leo*) and tigers (*Panthera tigris*) at Safari Zoo Lahore, focusing on the effectiveness of environmental modifications in reducing stress-induced behavior and physiological responses. Utilizing the focal method for behavior monitoring across three phases pre-pandemic, post-pandemic, and enrichment each spanning 15 days for a total of 45 hours of observation, the study examined the normal passive, normal active, and stereotypic behavior of 32 lions and five tigers. Enrichment interventions included the introduction of catnip and prey dung in enclosures. Cortisol levels were non-invasively measured using ELISA from fecal samples to assess stress. Key findings revealed a significant decline in stereotypic behaviors and cortisol levels post-enrichment, indicating an enhancement in animal welfare. Post-enrichment observations showed a notable decrease in stereotypic pacing in lions by 40% (±5%) and a reduction in cortisol levels by an average of 20% (±2%), showcasing the positive impact of sensory enrichment on captive big cats. The study also explored visitor effects on animal stress, finding a correlation between reduced visitor numbers and lower stress markers during the pandemic, suggesting that visitor presence significantly impacts stress levels. The study underscores the pivotal role of sensory enrichment in improving the quality of life for captive big cats by reducing stress-related behaviors and physiological markers of stress. These findings highlight the importance of implementing species-specific enrichment programs in zoos and similar conservation settings to promote the psychological and physical health of endangered species in captivity. The utilization of non-invasive methods like ELISA underscores the potential for ongoing welfare assessments without adding to the animals' stress, supporting a shift towards enriched environments as a standard in captive animal management and a re-evaluation of traditional practices in zoological settings.

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INTRODUCTION

Wild animals are brought into captivity for many reasons conservation, research, and recreation. While the physical needs of animals are met in captivity, the conditions of confinement and exposure to humans can result in physiological stress. The stress response consists of the suite of hormonal and physiological reactions to help an animal survive potentially harmful stimuli.

However, captivity may have long-term or permanent impacts on physiology if the stress response is chronically activated and leads neurosis. Research has shown that an animal's welfare is highly dependent on how various individual animal factors (e.g., species traits, genetics, temperament and previous experience) interact with environmental features (e.g., social grouping, enclosure design and sensory environment). One prominent feature of a zoo's environment is the presence of visitors. Decades of research on the visitor effect in zoos has demonstrated that visitors can have negative, neutral or positive impacts on zoo animal behaviour and welfare. According to Suárez *et al*. (2017) the influence of visitors on the welfare of captive animals, known as the visitor effect, may in some instances be stressful, adversely affecting animal health. Captive animals exhibit stereotypic behavior in response to multiple causes, including the inability to escape from human contact. The animal just needs something to occupy its mind with doing. This is known as enrichment. The enrichment techniques can improve harmful abnormal and stereotypic behavior arising in captive wildlife with increasing environmental complexity (Fischer and Romero, 2019).

In the current research work olfactory stimulation is applied in enclosures of captive lions (*Felis leo*) and tigers (*Felis tigris*). Olfactory enrichment refers to the addition of scents or scented material to an animal's enclosure (Young, 2013; Elwell and Vaglio, 2023). Typical enrichment items include food scent, essential oils, herbs and spices, feces, urine and other scents derived from animals, commercial lures, and artificial scent (Clark and King, 2008). The evidence from clinical trials shows the promising therapeutic effect of aromatherapy in reducing depressive symptoms (Sánchez-Vidaña *et al*., 2017). Success has been found in olfactory stimulation via the introduction of novel scents (Antonenko *et al*., 2019). Suarez *et al*. (2017) also found olfactory stimuli suitable to minimizing stereotypies irrespective of particular traits. Scent-based enrichments can be effective at increasing active behaviours in captive animals and potentially improve their welfare (Vaglio *et al*., 2021).

The study was carefully planned to evaluate the impact of visitor presence before the COVID-19 era and their absence during the COVID-19 pandemic on the behavior of Lahore Zoo Safari's main animals, specifically lions and tigers. According to Fàbregas *et al*. (2012) observation, these magnificent animals are famous landmarks because of the way they enchant tourists with their striking predatory tendencies and breathtaking good looks. In addition, the study investigates the effects of a brief olfactory enrichment program on levels of cortisol in feces, using the Enzyme Linked Immunosorbent Assay (ELISA). The objective of this project is to provide a simple and efficient stress assessment tool, which will help improve the health, well-being, and conservation efforts for captive big cats. Significantly, every cohort of lions and tigers alternated spending one week in an exposed safari environment, and the Lahore Zoo Safari administration was asked to be gracious and ensure consistent living arrangements for the participant carnivores during the whole research period.

MATERIALS AND METHODS

Study site

Lahore Safari Zoo was chosen as the study site. The lion and tiger safaris were divided into two sections i.e., four display cages (concrete/artificial) in front of both safaris and open safari (semi-natural). Each display cage measured 20×25 feet (46.45 m^2) while size of both open safaris was 15 acres (6.07 ha).

A group of lions was also housed near veterinary hospital in a small but semi-natural enclosure having area of 20×50 feet (92.9 m^2).

Study animals

Thirty two genetically related lions divided into seven groups and five tigers in three groups were studied during this research period. Safari management already did the grouping of lions and tigers. Three groups of tigers were from different origin and kept in different enclosures. A pair of tigers (UAE-Tigers) in group-1 was brought from UAE to change bloodlines in order to avoid inbreeding depression and housed in the front display enclosure. These tigers were originally pets. The group-2 (BWP-tigers) contained a tiger pair that was brought from Bahawalpur Zoo. In group-3 (T-white) only a solitary white tiger was present born in safari was housed in open tiger safari due to its furious habit. Table I shows the details regarding housing conditions of lions and tigers.

Lions were divided into three age groups Adult > 3 years, sub-adult 15 months–3 years old, and cub < 15 months, while all tigers under study were three years old. Table II shows the details of gender, age, health status of all lions and tigers under observation.

The study animals on average were fed with seven kg meat (fresh beef) in summers and nine kg meat in winter for six days a week. The subjects were also given some occasional treat (chicken) once in the week.

Focal method of behaviour monitoring and inferences drawn

The behavior of study animals was observed through focal method of monitoring animal's behavior

Table I. The housing details of 07 groups of lions and three groups of tigers.

Animal groups	Enclosure location	Conditions			
		Natural artificial	Pool	Access to visitors	Retreat access
Lions					
Group-1	Veterinary hospital enclosure	Artificial with vegetation and sun exposure	✓	Less access to visitors	✓
Groups- 2, 4, 5, 6, 7	Front display enclosures	Artificial (cement floor and iron bars. No vegetation only wooden logs present)	✓	High access to visitors	✓
Group-3	Lion open safari	Semi-natural with heavy vegetation cover and sun exposure	✓	High access to visitors through bus	✓
Tigers					
UAE Tiger-1 BWP Tiger-2	Front display enclosures	Artificial (cement floor and iron bars. No vegetation only wooden logs present)	✓	High access to visitors	✓
White Tiger-3	Tiger open safari	Natural and vast with tress, heavy vegetation cover and sun exposure.	✓	Visitors access through visitor bus	✓

Table II. Gender, age, and health status of lions and tigers.

Animal groups and members	Gender, age, health	
	Males (n)	Females (n)
Lions		
Group-1 (10 members)	4 Males (1 adult healthy, 2 sub adult healthy, 1 cub (crippled))	6 Females [1 adult healthy, 2 sub adult, (healthy 01+ crippled 01), 3 cubs (crippled)]
Group-2 (2 members)	1 Male (adult healthy)	2 Females (adult healthy)
Group 3 (8 members)	3 Males (adult healthy, 1 sub adult healthy, 1 cub healthy)	5 Females (3 adult healthy, 1 sub adult healthy, 1 cub healthy)
Group-4 (3 members)	1 Male (adult healthy)	2 Females (adult healthy)
Group-5 (2 members)	1 Male (adult healthy)	1 Females (adult healthy)
Group-6 (3 members)	1 Male (adult healthy)	2 Females (adult healthy)
Group-7 (4 members)	1 Male (adult healthy)	3 Females (adult healthy)
Tigers		
UAE tiger-1	1 Male	1 Female
(2 members)	Adult healthy	Adult healthy
BWP tiger-2 (2 members)	1 Male (adult healthy)	1 Female (adult healthy)
White tiger-3 (1 members)	1 Male (adult healthy)	N.A

(Schaller, 1972). Table III shows the ethogram of observed behaviors along with description as normal behavior (active behaviors and passive) stereotypic behaviors.

The behavior monitoring of animals was done from February 2020 to April 2020 during COVID-19 pandemic in three phases:

Pre-enrichment phase 12th February to 26th March, enrichment phase (27th February to 4th March and 12th to 18th March) consecutive by 30 for days without any gap (15 days in each phase) which was done pandemic phase (1stApril – 16th April) done for 15 days with gap of two weeks from enrichment phase. During all phases the time for monitoring was five hours/day from 11:00 am to 4:00 pm. The captive felids are usually diurnal, as they inhabit indoor cages during night. Due to this reason they are more active at midday and feeding periods (Babitz et al., 2023). The behavioral data collected from study was used to calculate the frequency and prevalence of different behaviors (Lucy and Newton-Fisher, 2004).

$$\text{Frequency of observed behaviors} = \frac{\text{Number of times a behavior is recorded for an animal}}{\text{Total number of observations for that animal}}$$

$$\text{Prevalence of Stereotypic Behavior} = \frac{\text{Number of individuals performing the behavior}}{\text{Total number of Individuals}}$$

Table III. Ethogram of recorded behaviors in lions and tigers.

S. #	Behavior	Description
Stereotypic behavior		
1	Pacing	Walking around a specific route in a cage without a purpose or destination.
2	Unusual sounds	Making sounds other than normal roaring. These may be growling sounds or shrieking sounds showing pain or frustration of the animal.
3	Aggressive ness	Unusual anger display and roaring.
4	Head tossing	Shaking head aberrantly and repeatedly due to anger and boredom.
5	Over grooming	Licking body fur beyond the level of cleaning or grooming resulting in removal of fur from parts of body.
6	Automutilation	Self-injury or self-harm by smashing or rubbing body with the bars of the cage or with the walls showing frustration.
Normal passive behavior		
1	Lying	Relaxation position of the animal showing a passive, less energy phase.
2	Sleeping	Natural rest state of unconsciousness of animal.
Normal active behavior		
1	Playing	Chasing, wrestling, pawing, stalking and rushing are common play behaviors. Cubs jump on females, play with tails, wrestle each other and roll on their backs. Solitary plying, acrobatic move, playing with branches/wood logs, running
2	Stretching	Act of physically reaching out of limbs showing fatigue from being in one position and also comfort in the space.
3	Social activity	Lions of parental groups called “prides” contain heritably associated females and cubs and they communicate using sound, touch and pheromones.
4	Grooming	Brushing or cleaning the coat of the animal by licking or using paws to remove dirt etc. from the body.
5	Climbing or digging	Rising up to a higher position in a playful manner.
6	Territory marking	Leave urine and scat, and scrape the ground to mark territory using their scent Urine marking mostly performed by males however females occasionally spray.
7	Communication	Body touching and sitting together, food offering, foraging together, lip smacking, chasing/moving together

Olfactory enrichment plan

The biological scents (catnip and prey dung) were applied in enclosures of subjects as short-term olfactory enrichment plan (Clark and King, 2008).

Catnip (*Nepeta catariale*, petmania.com) was spread in the measure of 20g per animals on floor of enclosures where lions of group-2, 4, 6 and 7 and tigers of group-1 and 2 were housed (Sconfienza *et al*., 2015). Five small cloth bags having 50g of catnip in each were hidden at different areas near favorite places of lions of group- 1 and 3 and for tiger of group- 3 in open yard/safaris. The catnip was provided for one week during enrichment phase.

As big cats can recognize their natural predators by olfaction due to their innate ability, in present research work zebra (*Equus burchellii*) dung collected from ungulate section of safari was used for olfactory enrichment. About 600g of zebra dung was hidden at different locations in open yard/safaris for group 1-3 of lions and group-3 of tigers. The group-2, 4, 6 and 7 of lions and tigers of group-1 and 2 were also enriched with 300g of prey dung hidden under wood log present in display enclosures. Prey dung was offered to all groups for seven days just after a week of catnip enrichment. Thus, the animal had space for stalking and showing other predatory behavior.

Fecal collection, preservation and ELISA processing

Fecal samples were collected according to Bashaw *et al*. (2016) and Yarnell *et al*. (2016). Four samplings were carried out using a pooled fecal technique, following the methodology described by Creel *et al*. (2013). The first sampling took place following the pre-enrichment phase, while the second sampling was done after the introduction of catnip enrichment. Similarly, the third sampling occurred towards the end of prey dung enrichment, and the fourth on the last day of the pandemic phase. Surveillance of lion and tiger enclosures was conducted every three hours from 06:00 to 24:00 h for three consecutive days to collect new fecal samples. Three new samples were obtained from each lion cage in groups 2, 4, 5, 6, and 7, as well as from each tiger cage in groups 1 and 2. Each day, a total of 21

samples were homogenized and mixed, combining three samples from each cage into a single plastic bag. A total of 21 primary samples were obtained on the designated sampling days, which consisted of seven groups kept in concrete cages. The freshness standards were established based on the silky texture, glossy dark brown hue, warmth, and enduring aroma. In addition, five new fecal samples were collected every day from different areas in the open yard of lion group-1 and -3, as well as tiger group-3. By utilizing the homogenization and mixing technique, a grand total of 15 samples were acquired from the lion and tiger safaris. Samples were preserved and processed.

The samples stored at -20 °C. The samples were thawed at room temperature and crushed after dehumidification at 60°C. Then 0.5g of every crushed sample was dissolved in 15 ml of 80% methanol, vortexed for 10 min, centrifuged for 15 min at 2000 rpm to pellet the sample.

The supernatant was used for the estimation of fecal cortisol levels by following Yarnell *et al*. (2016) and Bashaw *et al*. (2016) using by ELISA Kit Calbiotech (A Life Science Company).

Statistical analysis

In the comprehensive analysis conducted using OriginPro2022, a paired comparison plot with Tukey's method was generated to discern the prevalence of stereotypic behavior among lions and tigers. Employing pearson correlation, we further investigated the nuanced relationship between the number of visitors and the cortisol concentrations. This analytical approach evaluated the impact of environmental factors on big cat behavior and cortisol levels, shedding light on potential stressors in relation to visitor interactions.

RESULTS

Behavior monitoring

Lion behavior

The findings of mean frequency of observed behavior in lions housed at enclosure with small outer paddock near veterinary hospital (group-1) is given in Figure 1A. The normal active and passive behavior of this group was observed similarly like lion groups housed in front display cages of lion/tiger safaris. There was an overall increase in normal age-wise and phase-wise passive behavior. The order of sleeping and lying was adults > subadults > cubs. This gradually increased in all phases like pandemic phase > postenrichment phase > pre-enrichment phase. In adults the mean frequency normal passive and active behaviours was 0.086±0.001 in pre-enrichment, 0.103±0.001 in postenrichment and 0.116±0.001 in pandemic phase. It was 0.062±0.002 in preenricment, 0.073±0.001 in post-enrichment and 0.94±0.005 in pandemic phase in subadults. In cubs it was 0.048±0.001 in pre-enrichment, 0.053±0.000 in post-enrichment and 0.59±0.005in pandemic phase. In Figure 1B the normal active behaviour of lions is also explained. It was increasing oder age wise while these behaviours were less in pre-enrichment phase and approximately increased and got equal in post-enrichment and pandemic phase. In pre-enrichment phase it was 0.154±0.002 in adults, 0.215±0.001 in subadults and 0.243±0.003 in cubs. It was 0.204±0.003 in adults, 0.315±0.002 in sub-adults and 0.295±0.001in cubs in enrichment phase and 0.204±0.002 in adults, 0.283±0.002 in sub-adults and 0.350±0.004 in cubs during pandemic phase.

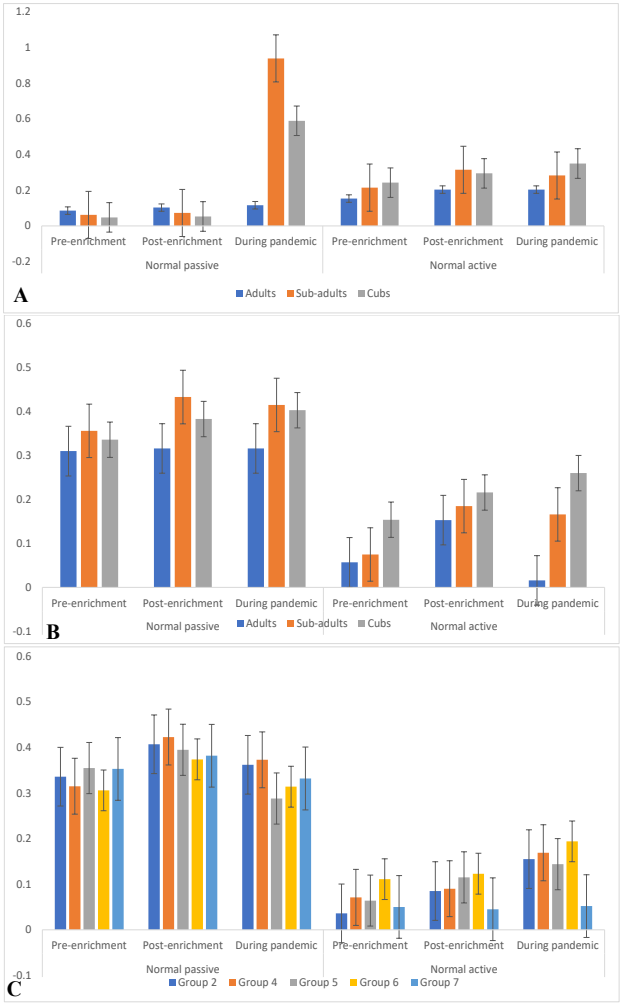

Fig. 1. Mean frequency of observed behaviors in lion group 1 (A), lion group 3 (B) and lion groups -2, 4, 5, 6, 7 (C). ** groups -2, 4, 5, 6,7 lion housed at concrete enclosures in front of lion/tiger Safari.

The findings of mean frequency of observed behaviors in lions housed at enclosure with small outer paddock near veterinary hospital (group-3) is given in Figure 1B. The normal active and passive behavior of this group was observed similarly like lion groups housed in front display cages of lion/tiger safaris. There was an overall increase in normal in age-wise and phase-wise. passive behavior. The order of sleeping and lying was adults >subadults > cubs. This gradually increased in all phases viz. pandemic phase > post-enrichment phase >pre-enrichment phase. The order of sleeping and lying was adults > sub-adults > cubs. This gradually increased in all phases *viz*. pandemic phase > post-enrichment phase > pre-enrichment phase. In adult the mean frequency normal passive and active behaviour was like 0.310±0.002 in pre-enrichment, 0.316±0.001 in post-enrichment and 0.316±0.101 in pandemic phase in adults. It was 0.356±0.001 in pre enrichment, 0.433±0.001 in post-enrichment and 0.415±0.001 in pandemic phase in subadults. In cubs it was 0.336±0.001 in pre-enrichment, 0.383±0.002 in post-enrichment and 0.403±0.001in pandemic phase in cubs. In Figure 3 the normal active behaviour of lions is also explained. It increased in order age wise while these behaviours were less in pre-enrichment phase and approximately increased and equaled in post-enrichment and pandemic phase. In pre-enrichment phase is was 0.057±0.019 in adults, 0.075±0.002 in sub-adults and 0.154±0.002 in cubs. It was 0.153±0.303 in adults, 0.185±0.002 in sub-adults and 0.216±0.001 in cubs in post-enrichment phase and 0.016±0.001 in adults, 0.166±0.002 in sub-adults and 0.260±0.002 in cubs during pandemic phase.

The Figure 1C is presenting mean of normal passive and normal active behaviours of lions groups- 2,4,5,6,7 housed in frnot cages of lion safari. The mean frequency of normal passive category is mostly similar in pre-enrichment phase ranging from 0.306±0.001 to 0.355±0.001. In post-enrichment phase it further increased up to 0.423±0.002 in group-2. In the absence of visitors during pandemic phase a prominent decrease 0.288±0.001 was observed in group-5. It is also obvious from Figure 1 that the activity level of all lion groups was less in pre-enrichment phase and was noticeably increased in pandemic phase. The order of activity level increase was pandemic phase > post-enrichment phase > pre-enrichment phase. During pre-enrichment phase it ranged between 0.036±0.002 and 0.111±0.001, during post-enrichment the highest level of activity was observed in group-6 that is 0.123±0.002 but in pandemic phase an overall increase of active behaviours was present in all lion groups ranging from 0.052±0.002 to 0.194±0.002.

Tiger behavior

Figure 2 presents the mean of normal passive and normal active behaviors of tigers in group- 1 and 2 housed in concrete enclosures in front of tiger safari and group 3 housed at the open safari. The mean frequency of the normal passive category in the pre-enrichment phase was higher in group 2 0.312±0.001 and lower in group 1 0.289±0.005. In the post-enrichment phase, it was further increased up to 0.399±0.005 in group 2. In the absence of visitors during the pandemic phase, a prominent decrease of 0.312±0.001 was observed in group 1. It is also obvious from Figure 1A that the activity level of all tiger groups was less in the pre-enrichment phase and was noticeably increased in the pandemic phase. The order of activity level increase was pandemic phase > postenrichment phase > pre-enrichment phase. During pre-enrichment, it ranged between 0.022±0.001 to 0.051±0.001, during post-enrichment the highest level of activity was observed in group 1 that is 0.068±0.004 but in the pandemic phase an overall increase of active behaviors was present in all tiger's groups ranging from 0.074±0.004 to 0.103±0.003.

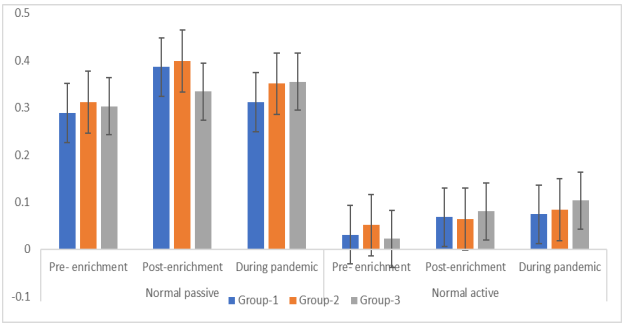

Fig. 2. Mean frequency of observed behaviors in tiger groups 1, 2 and 3. *Pre -enrichment is also the pre-pandemic phase. ** groups -1 and 2: Tigers housed at concrete enclosures in front of tiger safari. *** group-3: Tiger housed at open safari. For pre-enrichment prevalence of stereotypical behavior differs significantly from pandemic prevalence. Post-enrichment stereotypic behavior differs from pandemic behavior in a relatively significant way. P-values (p=0.05) are indicated with asterisks (*). The means and standard errors are depicted by bars for each treatment.

Prevalence of stereotypic behavior in lion and tiger

Lions

The overall trend in all lion groups indicates that enrichment activities reduce stereotypic behavior in most lion groups. However, the extent of this decline and the response to enrichment varied significantly between groups like significance level of group 7 was 0.134±0.002 and group 1 was 0.045±0.002, and external circumstances, such as the pandemic, may influence behavior differently in each group. Groups 6 and 7 appear to be less affected by

enrichment. However, groups 3 and 1 had a more moderate reaction to enrichment and exhibited more stereotypic behavior during the epidemic than other groups.

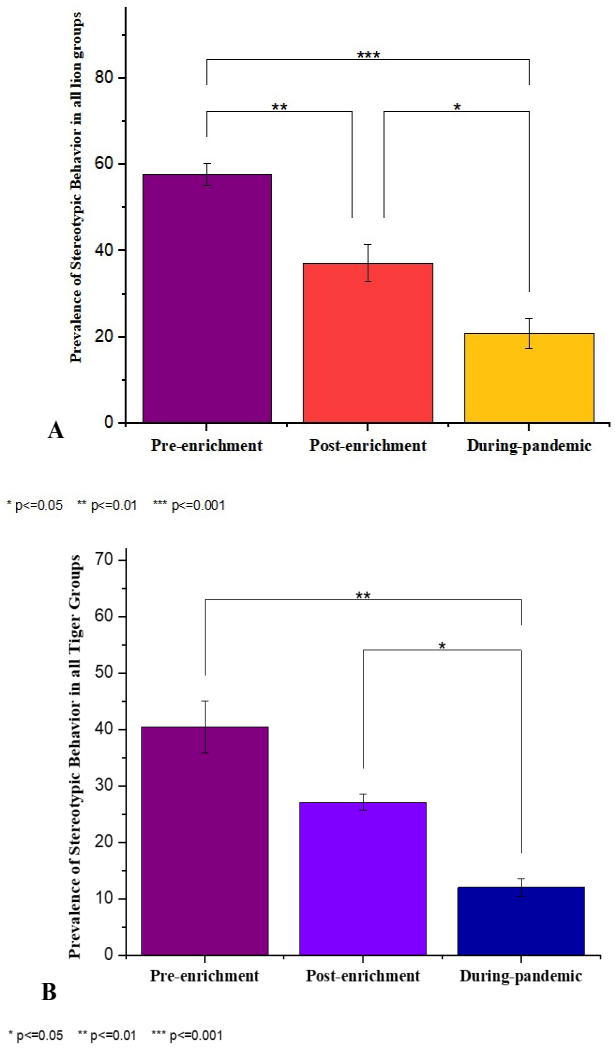

Fig. 3. Prevalence of stereotypic behavior in all lion groups (A), and tiger groups (B). During pre-enrichment, the prevalence of stereotypical behavior is substantially lower than during the pandemic. During the pandemic, the results revealed a marked improvement in the behavior of lions of these groups. The significant improvement in the behavior of lions during the pandemic is due to a less number of visitors to the zoo, which reduces their stress levels. P-values (p=0.05) are indicated with asterisks (*). The means and standard errors are depicted by bars for each treatment.

Tigers

Figure 3 illustrates the prevalence of stereotypic behavior across three conditions or time points: Pre-enrichment, post-enrichment, and during-pandemic. Animals who engage in stereotypical behavior frequently exhibit odd, repetitive actions that can indicate stress or boredom. After the enrichment, there is a consistent trend of decreasing prevalence of stereotypic behavior across all three groups. This indicates that enrichment positively affected all groups in reducing these behaviors. When proceeding from the post-enrichment condition to the during-pandemic condition, there is a general trend of continued declines in the frequency of stereotypic behavior. In contrast, group 2 shows a lesser decline during the pandemic; this group showed more significant difference.

Fecal cortisol concentration in lions and tigers

Lions

Figure 4 illustrates the levels of cortisol concentration (expressed in ng/ml) observed in different lion groups, namely pre-enrichment, post-enrichment with catnip, post-enrichment with prey dung, and during the pandemic. The cortisol concentration during the pandemic ranged from 188.166±0.440 (group 7) to 90±0.577 (group 1) and in pre-enrichment 323±1.527 to 191.33±0.881. There was a significant difference in cortisol levels during the pandemic and the pre-pandemic, specifically when there were no visitors. The effects of enrichment with catnip and prey dung on cortisol concentration and stress level are comparable, due to their significant difference with pre-enrichment.

Tigers

The cortisol concentrations observed in all tiger groups, namely pre-enrichment, post-enrichment with catnip, post-enrichment with prey dung, and during the pandemic has been illustrated in Figure 4. The cortisol concentration during the pandemic significantly decreased in all groups. There was a significant difference in cortisol levels during the pandemic and the pre-pandemic, specifically when there were no visitors. The effects of enrichment with catnip and prey dung on cortisol concentration and stress level were also comparable.

Correlation between cortisol concentration and number of visitors

Lions

The correlation between cortisol concentration in all lion groups and number of visitors (pre-enrichment, post-enrichment and during pandemic) is displayed in Table IV. In pre-enrichment the cortisol level in all lion groups is positively correlated with number of visitors indicating that with the increase in number of visitors the cortisol level increases in all the lion groups. In post-enrichment

the cortisol level in all lion groups is negatively correlated with number of visitors indicating that enrichment has significant effect on cortisol concentration. The cortisol level during the pandemic was significantly reduced as there were no visitors.

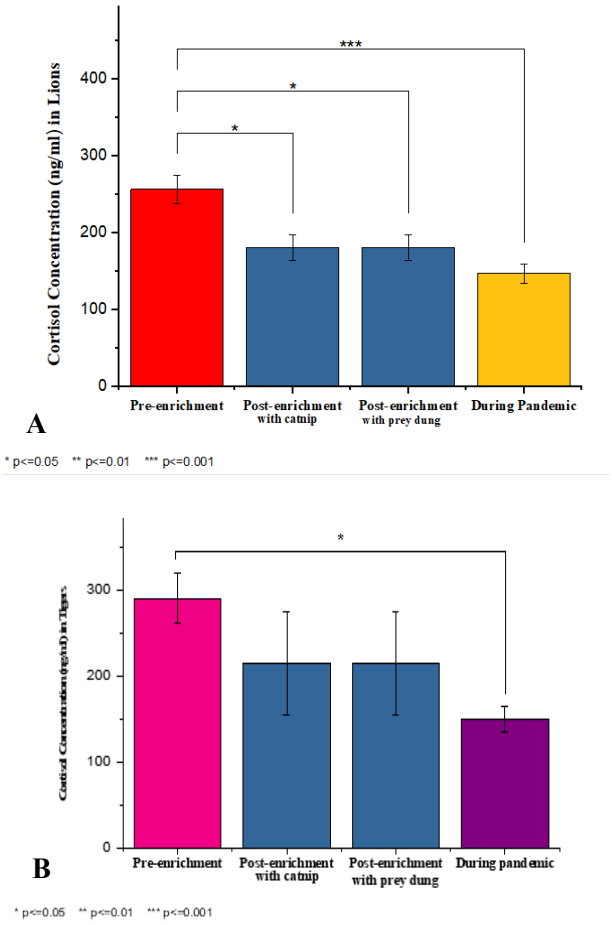

Fig. 4. Cortisol levels before and after enrichment (with prey dung and catnip) and during the pandemic in all the lion groups (A) and tiger groups (B). There is a moderately significant difference between pre-and post-enrichment (with catnip and prey dung) in the presence of visitors and a highly significant difference between pre-enrichment and during the epidemic (the absence of visitors). P-values are indicated with asterisks (*) (p 0.05 and p 0.001). The means and standard errors are depicted by bars for each treatment.

Tigers

The correlation between cortisol concentration in all tiger groups and number of visitors (pre-enrichment, post-enrichment and during pandemic) is displayed in Table IV. In pre-enrichment the cortisol level in all lion groups is positively correlated with number of visitors indicating the cortisol level increase with the increase in number of visitors. In post enrichment, the cortisol level is positively correlated with number of visitors, indicating that enrichment significantly affects tigers. The cortisol level during the pandemic was significantly reduced as there were no visitors during pandemic.

Table IV. Pearson correlation coefficient (r) between cortisol concentration in all the lion and tiger groups and number of visitors (pre-enrichment, post-enrichment, and during pandemic).

Label	Pre-enrichment	No. of visitors	Post-enrichment	No. of visitors
Lion				
Pre-enrichment	1			
No. of visitors	0.40635	1		
Post-enrichment	0.67626	-0.74612	1	
No. of visitors	-0.47191	0.98898	-0.75438	1
During pandemic	0.61184	-0.88569	0.91935	-0.88931
Tiger				
Pre-enrichment	1			
No. of visitors	0.47357	1		
Post-enrichment	0.76788	0.92785	1	
No. of visitors	0.47357	1	0.92785	1
During pandemic	0.98725	0.32734	0.65612	0.32734

DISCUSSION

The study delves into the behavioral characteristics of lions and tigers in captivity, focusing on how enrichment strategies, particularly catnip, impact their behavior and stress levels. The researchers aim to understand the role of natural selection in shaping these behaviors and emphasize the importance of environmental factors.

According to the enrichment plan, the catnip was given with the gap of one day. Lions sniffed and smelled around the entire cage in the anticipation of finding catnip. Tigers however, did not show that much interest. As the enrichment plan was short-term, there was a possibility of increased stress as an animal struggles to get used to something new. Some causes of stress in lions and tigers at Lahore Safari Zoo (Fig. 5) include environmental conditions such as enclosure size, temperature fluctuations, and lack of natural habitats.

The frequency and prevalence data were interpreted in the form of graphs showing the improvement in animal behavior and exhibition of stereotypic behaviors through enrichment. To check if enrichment had an impact on the cortisol levels, paired sample T-test was smeared to the

readings gained since pre-enrichment samples and post-enrichment samples.

Table V. Visitors statistics at Lahore Safari Zoo.

Days	No. of visitors	Animal to visitors ratio	
		Lions	Tigers
Lahore Safari Zoo			
Working days	1000	1:31	1:42
Week ends	6000	1:187.7	1:857
Public holidays	8000-10,000	1:313	1:1428
During pandemic	0 visitors	Only safari staff	
Bus tours in lion/tiger Safari			
Working days	10-15 buses (25-30 persons/bus)	1:10	1:450
Week ends	20-30 buses (25-30 persons/bus)	1:90	1:900
Public holidays	30-50 buses (25-30 persons/bus)	1:150	1:1500
During pandemic	No bus tour	Only safari staff	
Front display enclosures of lion/tiger safari			
Working days	100-200	1:9	1:33
Week ends	300-500	1:22	1:83
Public holidays	500- 1000	1:45	1:166
During pandemic	0 visitors	Only safari staff	
Visitors hospital enclosure			
Working days	50-80	1:6	
Week ends	100-130	1:11	
Public holidays	150-180	1:16	
During pandemic	0 visitors	Only safari staff	

Catnip as enrichment

Catnip was used in enrichment plans for cats for a long time. In 2011, it was used as part of an enrichment plan along with cinnamon for leopards (Resende *et al*., 2011). According to this study, odor of catnip was not helpful in reducing the stereotypic pacing in these leopards. However, in the present study, when the catnip was scattered on the floor of the enclosure, the animal showed curiosity and later sniffed and rubbed it's body on it. The display of stereotypic behaviors reduced significantly and even the day after use, the animal was expecting to receive enrichment. Hence, the behavior showed anticipation and the lions even searched the entire enclosure in hopes of finding catnip.

In another enrichment plan devised for tigers in 2014 (Myatt, 2014), catnip was used. A significant increase in positive behaviors like grooming, sniffing and playing was observed. Also, the fecal cortisol levels reduced significantly from before enrichment (150-280 ng/mL) to after enrichment (below 150 ng/mL). These results agree with our findings.

Lions

Lions under study were studied once in 2018 (Khan *et al*., 2018) in comparison of lions in Lahore Zoo and behavior was divided into two categories (natural and anomalous). The results showed that abnormal behavior patterns were shone by all captive lions. According to our studies, the frequency and prevalence of stereotypic behaviors of lion groups in seminatural enclosures (with natural substrate, vegetation and sun exposure) which were lion group-1 and lion group-3 were lesser than those living in artificial enclosure (concrete floor, no vegetation or sun exposure and iron bars) which were lion group 2, 4, 5, 6 and 7. The male of group 5 showed little response to the enrichment behavior but it could also be because it was kept solitary in the cage while the female of this group was not given catnip because she was pregnant and about to give birth at the time of enrichment. Lions are used to being social so being kept solitary must have caused it to respond less to catnip.

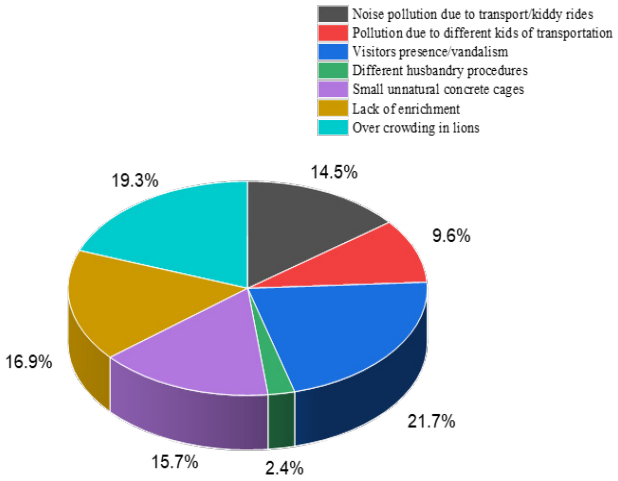

Fig. 5. Causes of stress in lions/tigers at Lahore Safari Zoo.

It is evident from results of the mean frequency of normal active and passive behavior in lion groups -2, 4, 5, 6, 7 that lions are highly affected by the presence of visitors and the area of the cage and its structure are vital component of catnip wild lions. The findings also indicates that the short-term aroma therapy improves the activity level of lions, but the results are more significant in the pandemic. The lions in front cages of lion/tiger safaris are highly visited by the public every day as shown in Figure 6. The lions to visitor ratio is 1:9 which is almost equal

to lions living in open safari with big area that is 1:10. The main reason for this large crowd is the popularity and easy approach of the lion/tiger safari. At these cages the public is very close to lions, other than over-crowding of visitors the different kinds of animal rides, canteen, aviary and electronic rides are also badly affecting the lions. This is the reason that the lying and sleeping is very prominent in every lion group mentioned in Figure 1. All these lion groups were displayed more passive behaviour than active behaviour as mentioned earlier, the factors may be responsible for this: concrete cages and overcrowding with visitors.

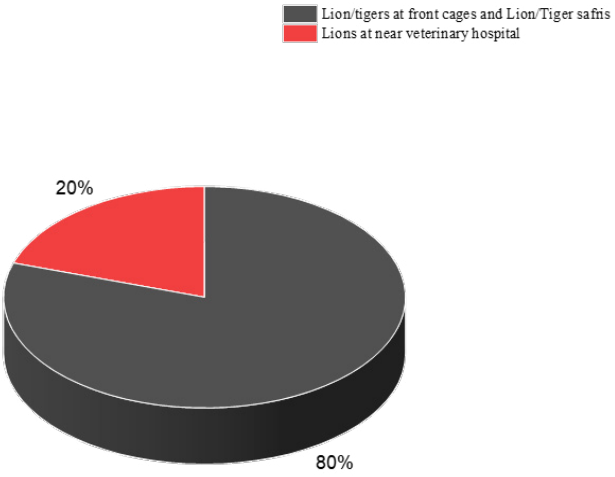

Fig. 6. Percentage of vistors time at different locations of Lions and tigers at Safari.

The details of lion housing conditions and details of gender and age are given in Table I living in veterinary block. In general, the cubs are more playful and active as compared to adults; this is the reason for the passive behaviour order adults > sub-adults > cubs in lions of group-1. On the other hand, the adults just to avoid the public and disturbance spend more time in sleeping and lying. Even if the adults are lying the cubs are used to playing with their parents. The normal active behaviours like playing, stretching, social activity, grooming, climbing or digging, territory marking, communication were improved in enrichment and pandemic phases. The adults and sub-adults were more involved in stretching, grooming, territory marking, and communication. While the cubs were more playful and socially active. The cubs were highly active during pandemic phase while the sub-adult were notice more busy in enrichmnet phase but the level of activity was similar in adults during enrichment and pandemic phases. The reason is the elevation of sensory stimulation by aroma therapy with enrichment and least disturbance due to absence of visitors. This group of lions are facing less visitors stress as lions to vistiors ratio was 1:6 on working days that is 30% less than other groups of lions. Similarly, due to decreased visitors the disturbance created by them was also less and different types of rides were not there. Other than these factors this cage was less available because it was away from the main entrance and visitors spend 80% of their time at lion and tiger safaris. Presence in family groups along with cubs also reduced the stress.

Tigers

It is evident from the results of the mean frequency of normal active and passive behavior in tiger groups 1, 2, and 3 that tigers were significantly affected by the presence of visitors and the area and structure of the enclosure, which is a vital component of catnip tigers. The findings also indicated that the enrichment had increased the tigers' activity level, but the results were more significant during the pandemic. As shown in the Table V, the tigers (group 1 and group 2) in the front enclosures of tiger safaris are visited frequently every day. The ratio of tigers to visitors is 1:33, which is significatly higher than the ratio of tigers living in an open safari with a large area, which is 1:45. This large crowd is primarily due to the popularity and accessibility of the tiger safari. In addition to overcrowding, the various animal attractions, canteen, aviary, and electronic rides have a negative impact on the tigers. Sleeping and lying down were more prominent in the tiger group. As previously stated, these tiger groups demonstrated more passive than active behavior. These reasons may be responsible due to the concrete cages and overcrowding with visitors. This supports the assumption that habitats close to the natural habitat of animal are less stressful to the animal as quoted in the book Zoo Biology (Fàbregas et al., 2012). These data also agree with the studies done on leopards and tigers in India in 2017 which showthat the size of the field is one of the most impelling aspects of stereotypic behavior, as tigers kept in fields smaller than the size prescribed by CZA stereotyped higher than those in larger enclosures (Vaz *et al*., 2017).

Prevalence of stereotypic behavior

The prevalence of stereotypic behavior in lions and tigers in captivity varies among groups. In lions, enrichment activities generally reduce stereotypic behavior in most groups, but the extent of this decline and the response to enrichment differed significantly between groups, and external circumstances, such as the pandemic may influence behaviour differently in each group. Groups 3 and 1 had a more moderate reaction to enrichment and exhibited more stereotypic behaviour during the epidemic

than other groups. In tigers, there is a consistent trend of decreasing prevalence of stereotypic behavior across all three groups (pre-enrichment, post-enrichment, and during pandemic) when enrichment is applied. Group 2 showed a lesser decline during the pandemic, suggesting that this group may have been more affected by external factors. These findings indicate that enrichment is a promising technique for reducing stereotypic behavior in captive lions and tigers, but the effectiveness of enrichment may vary among groups and species, and external factors may influence the behavior (Mason *et al*., 2007; Vaz *et al*., 2017).

Fecal cortisol concentration in lions and tigers has been studied to assess the impact of enrichment and the prevalence of stereotypic behavior. In lions, cortisol levels during the pandemic ranged from 188.166±0.440 ng/ml (group 7) to 90±0.577 ng/ml (group 1) during pre-enrichment, and there was a significant difference in cortisol levels during the pandemic compared to pre-pandemic conditions. Enrichment with catnip and prey dung had comparable effects on cortisol concentration and stress level, as they significantly differed from pre-enrichment. In tigers, cortisol concentrations during the pandemic significantly decreased in all groups, and the effects of enrichment with catnip and prey dung on cortisol concentration and stress level were also comparable. These findings indicate that enrichment can positively affect cortisol levels and reduce stereotypic behavior in captive lions and tigers, but the effectiveness of enrichment may vary among groups and species, and external factors may influence the behavior differently in each group (Sarodeet al., 2019; Vaz *et al*., 2022).

Correlation between cortisol concentration and number of visitors

During the pandemic, cortisol levels in the majority of groups declined comparatively to their pre-enrichment levels. This could infer that despite the pandemic's disruptions, the lions experienced less stress. Group 1 and group 5 showed significantly decreased cortisol levels during the pandemic, indicating they were less stressed because group 1 was located away from the main entrance and visitors. However, group 7 demonstrated a slight increase in cortisol levels during the pandemic. As mentioned previously, concrete cages and overcrowding may be responsible for this increase in cortisol level. All tiger groups exhibited decreased cortisol levels during the pandemic compared to pre-enrichment levels. Group 2 and group 3 displayed significant decreases in cortisol level during the pandemic, indicating that the pandemic was a less stressful period for them. Group 1 also shows a decrease in cortisol levels during the pandemic, but the reduction is less pronounced than group 2 and group 3. The tiger groups exhibit consistent responses to catnip and prey dung enrichment, with both interventions leading to reduced cortisol levels. For all tiger groups, prey dung enrichment was more effective in reducing cortisol levels than catnip enrichment. The pandemic also had a stress reducing effect on the tigers, as indicated by the decreased cortisol levels during that period.

The study employed cortisol ELISA to measure stress levels in lions and tigers. Results indicate a correlation between the frequency of stereotypic behavior and cortisol concentration. The pandemic period, with reduced visitor interactions, corresponds to lower cortisol levels, suggesting a potential link between external stimuli and stress. The efficacy of enrichment, especially prey dung, in reducing cortisol levels further supports the positive impact of these interventions.

CONCLUSION

This study highlights how environmental enrichment can significantly reduce psychological discomfort and improve the well-being of caged large cats. Transitioning from sterile, restrictive environments to partially natural enclosures with plants and abundant sunlight resulted in a notable decrease in repetitive behaviour and stress level, as indicated by reduced levels of cortisol in faeces. Utilising catnip and zebra dung in enrichment efforts highlights the significance of tailoring captive settings to meet species specific preferences. The significant differences in the factors leading to negative outcomes in lions and tigers highlight the necessity for a comprehensive strategy to caring for animals in captivity. This study advocates for the use of realistic enclosures, personalised enrichment, and careful selection of scents to improve the well-being of these magnificent animals. This research urges zoo and safari experts, along with conservationists, to embrace the insights and advocate for a future where the physical and psychological health of caged species is the first priority. By implementing these enlightened care practices, we are moving towards aligning the lives of captive big cats with their natural needs, creating habitats where they can flourish rather than just exist.

DECLARATIONS

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IRB approval

The study has been conducted through a non –invasive method for the collection of samples without harming the animals due to this IRB approval not being taken.

Ethical statement

We read the policy of the journal on ethical consent and standard animal care, and our work was carried out in accordance with these policies

Statement of conflict of interest

The authors have declared no conflict of interest.

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