Effects of a combined enrichment intervention on the behavioural and physiological welfare of captive Asiatic lions (*Panthera leo persica*)

Sitendu Goswami[1*], Shiv Kumari Patel[1], Shwetank Pandit[2], Riyaz Kadivar[2], Praveen Chandra Tyagi[1], Pradeep Kumar Malik[1], Samrat Mondol[1*]

[1] Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand, India.

[2] Sakkarbaug Zoological Garden, Junagadh, Gujarat, India

[*] Corresponding authors: Samrat Mondol, Ph.D., Animal Ecology and Conservation Biology Department, Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand 248001. Email- samrat@wii.gov.in

Sitendu Goswami, Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand 248001. Email- sitendu@wii.gov.in

Running head: Impacts of enrichment on Asiatic lions.

Abstract

The endangered Asiatic lion (*Panthera leo persica*) is currently distributed as a single wild population of 670 individuals and ~400 captive animals globally. Although the captive lions are major hope for the species' long-term conservation through repatriation, their welfare status and management practises need research attention. To this end, we tested the efficacy of feeding, sensory and manipulable enrichment interventions on the welfare of Asiatic lions at the conservation breeding centre of Sakkarbaug Zoological Garden, Gujarat. We adopted a holistic approach by measuring physiological and behavioural responses of 35 captive Asiatic lions, divided into control (n=16) and test (n=19) groups. The test subjects approached feeding devices first and manipulable devices for a longer duration. Manipulable devices were used homogenously with two significant time peaks, but sensory devices were used sporadically throughout the day with no discernible peak usage. The control subjects remained unchanged in all welfare parameters compared to their pre-treatment levels. However, post-enrichment behavioural assessments showed higher behaviour diversity (95% increase from the baseline period), reduced enclosure zone bias (40.25% reduction) and aberrant repetitive behaviours (80.68%) in test samples. Similarly, faecal corticosterone measures showed lower stress levels in test samples (58% decrease), confirming significant improvement in all welfare indices than control groups. These results have universal applicability to assess welfare indices of other captive species in Indian zoos. We hope that the results will encourage zoo managers and regulatory agencies to improve animal welfare practices.

Keywords: Animal welfare, conservation breeding initiative, captive animal management, behaviour diversity, space usage, stereotypy, faecal corticosterone.

1. Introduction

Since the early Palaeolithic depictions of the "lowenmensch" by the Aurignacians (Chauvet et al., 1996), lions have been heralded across several cultures as emblems of man's relationship with nature (McCall, 1973). Once ubiquitous across south-western Asia between Syria and parts of eastern India (Ball, 1880; Joslin, 1984), they were hunted to extinction from large parts of their range by the late 19th century (Jhala et al., 2019; MacKenzie, 2017; Storey, 1991). During the 1860s, the Asiatic lion population in Gir forests, India was struggling to survive. Timely conservation measures along with a hunting ban were instrumental to the recovery of the species. Sakkarbaug zoo, founded in 1863, played a definitive role in the conservation of Asiatic lions by treating injured and diseased individuals and maintaining a viable captive stock for future repatriation. Subsequent affirmative conservation actions including an ex-situ conservation breeding programme (Smith, 1984) led to the species revival from the edge of extinction. Today the extant population of more than 500 wild Asiatic lions(Gujarat Forest Department, 2015) inhabit fragmented habitats scattered over a human dominated landscape in Gujarat(Gogoi et al., 2020), Western India, along with approximately 400 Asiatic lions spread across zoos in several countries, with more than 60% of the captive population residing in Indian zoos and conservation breeding centres (Srivastav et al., 2018). The survival and proliferation of this species relies on repatriation to insulate the extant population from future stochastic extinction events (Jhala et al., 2019). Although the conservation breeding programme has been successful in maintaining a genetically diverse stock, the welfare status and incumbent management practices for the animals has not received adequate research focus (Pastorino et al., 2017; Goswami et al., 2020).

Conservation breeding programmes for endangered species should be designed to promote species-typical behaviours and cognitive plasticity for better post-release fitness (Rabin, 2003).

71 The deleterious impacts of sterile captive environments manifest with loss of species-typical
72 behaviours and increase in psychosomatic disorders in animals(Broom, 2011; Dawkins, 2004;
73 Fraser, 1999; Rabin, 2003). Therefore, welfare-based management practices are vital for any
74 successful conservation breeding programme (Swaisgood, 2010). The benefits of an individual-
75 centric welfare evaluation (Joslin, 1984) complemented with targeted enrichment interventions
76 has been successfully demonstrated for several captive animals viz., ursids (Carlstead et al.,
77 1991; McGowan et al., 2010), felids (Powell, 1995; Suárez et al., 2017), canids (Cloutier and
78 Packard, 2014; Leonard, 2008), equids (Bulens et al., 2013), small mammals (Clark and Melfi,
79 2012; Vargas and Anderson, 1999), reptiles (DeGregorio et al., 2017), and amphibians (Michaels
80 et al., 2014). Apart from improving welfare, enrichment interventions have been shown to play
81 an important positive role in increasing post-release fitness in several species (Brown et al.,
82 2003; Rabin, 2003; Reading et al., 2013). Earlier study on welfare status of captive Asiatic lions
83 reported that individual variations (personality and rearing history) are associated with
84 differential welfare outcomes in lions housed under similar captive environments (Goswami et
85 al., 2020), necessitating individually tailored husbandry regimen for the animals. This study
86 focuses on identifying the areas for improvement of the current husbandry and management
87 practices for Asiatic lions.
88 Feeding, sensory and manipulable enrichments have been shown to improve the welfare of
89 captive felids (Powell, 1995; Van Metter et al., 2008). We tested the efficacy of a combined
90 enrichment intervention on the welfare of captive Asiatic lions at Sakkarbaug Zoological Garden
91 (SZG). We measured several behavioural (species-typical behaviour diversity, enclosure usage
92 and aberrant repetitive behaviours) and physiological (faecal corticosterone metabolites) welfare
93 indices as a response to enrichment interventions. This is the first controlled trial study to

94 incorporate both behavioural and physiological tools to measure the welfare status change in
95 response to enrichment interventions of Asiatic lions housed at a conservation breeding centre.
96 We hope that our findings assist in the improvement of incumbent husbandry and management
97 practices for the species.

98 **2. Methods**

99 ***2.1. Study area***

100 We conducted this study at the Asiatic lion conservation breeding centre at SZG. The
101 conservation breeding programme of Asiatic lions was initiated in 1958 with nine founders and
102 has since proliferated to house more than 47 individuals housed at SZG with several breeding
103 pairs in other participating zoos across the world. Since SZG houses both captive-born and wild-
104 rescued individuals, it holds intrinsic value for the conservation of Asiatic lions. During the study
105 period, the off-display conservation breeding facility housed 47 individuals in 20 large
106 naturalistic enclosures. A schematic map of the facility is provided in Supplementary Figure 1.

107 ***2.2. Subjects and housing***

108 We selected 15 enclosures housing 35 Asiatic lions for the present study. Some of the subjects
109 were wild-rescued (N = 19, Male = 11, Female = 8), while the rest were born in captivity (N =
110 16, Male = 3, Female = 13). Subjects were primarily adult animals (N = 31) with a few sub-
111 adults (N = 4) and were randomly assigned to test and control groups to ensure uniformity of
112 treatment. The test group consisted 19 subjects (Male = 7, Female = 12) the control group
113 consisted 16 subjects (Male = 7, Female = 9), respectively.

114 Asiatic lions were housed in 15 enclosures in pair (1:1 and 0:2) (N = 9) or heterosexual (1:2) (N
115 =6) configurations (Supplementary Table 1). All subjects were housed in the same enclosure for
116 at least a year with the same enclosure mate and were accustomed to the enclosure and

117 management practices. In most cases subjects were housed with related individuals. All
118 enclosures provided adequate space (above 400m^2/animal in general with some having
119 1500m^2/animal) for the animals. The naturalistic enclosures provided subjects with sufficient
120 natural vegetation cover, simulating the habitat of Asiatic lions while protecting subjects from
121 visitor disturbance. Enclosures were well covered with trees and shrubs, which provided subjects
122 with a good combination of shade and sun throughout the day. Fresh drinking water was made
123 available inside retiring cells adjoining the paddock area. Subjects were allowed free access to
124 the paddock area and retiring cubicles throughout the day and night. However, these enclosures
125 offered little in terms of novelty and cognitive enrichment. Our earlier study established low
126 enclosure space utilization, low behaviour diversity, and a high incidence of aberrant repetitive
127 behaviours prevalent in Asiatic lions housed at SZG (Goswami et al., 2020).
128 A group of 10 animal keepers and contractual workers carried out all husbandry work for the
129 lions. Keepers typically reported for duty at 0700 hours and cleaned all leftover food from
130 retiring cells during 0730-1100 hours. The leftover food items were weighed to ascertain the
131 food consumption by each individual. The keepers would provide the daily ration of buffalo
132 meat to the subjects between 1700-1800 hours at the feeding cubicle, where each lion was fed
133 separately. Keepers were also tasked with behavioural monitoring of the subjects and recorded
134 the commencement and cessation of mating events between subjects. The zoo managers and the
135 animal keepers met regularly to ascertain the welfare need of the captive animals based on
136 subjective evaluations. The approach to welfare assessment was preventative and based solely on
137 incidental keeper observations hence difficult to quantify and address. We expected to observe
138 the effect of novel enrichment devices on the behavioural and physiological welfare indices of
139 these captive animals.

140 ***2.3. Study design***

141 We hypothesized that enrichment interventions would lead to significant improvement in
142 behavioural (Goswami et al., 2020)and physiological welfare measures for all test subjects in
143 contrast to the controls. We divided the subjects into control (N = 16) and treatment (N = 19)
144 groups by a blind draw of enclosures for enrichment interventions. We conducted the study in
145 two phases: baseline and post-enrichment. During the baseline period, we measured behaviour
146 for 14 days/subject and collected two fresh faecal samples/subject/week. This was followed by
147 a one-week exposure to the enrichment intervention to get test subjects accustomed to the new
148 devices. During the testing period (14 days) we recorded the behaviour of test subjects as a
149 response to the daily enrichment intervention. Apart from the enrichment interventions, housing
150 and husbandry conditions remained unchanged for both groups throughout the course of the
151 study.

152 ***2.4. Data collection***

153 ***2.4.1. Behaviour observations***

154 Two observers (viz., first author and field assistant) collected all behaviour data, which
155 necessitated accounting for inter-observer reliability. We created a detailed ethogram of the
156 study subjects from one month of ad-libitum behaviour sampling and compared the behaviour
157 data recorded from the same subject by both observers to ensure consistency (Goswami et al.,
158 2020). We commenced data collection only after inter-observer reliability levels remained
159 consistent across three consecutive sessions (Cronbach's alpha >0.9). We simultaneously video
160 recorded all behaviour sessions to aid in data entry and reduce errors. We recorded behaviour
161 during 0500-1100, 1200-1800, and 2200-0500 hours. We used instantaneous scan sampling
162 (Altmann, 1974)at 1-minute intervals to record all behavioural states and all occurrences of

163 behavioural events (Goswami et al., 2020). During every six-hour data collection period, we
164 recorded four hours of behaviour data with 30 minutes rest for observers after every hour. Data
165 for the observer-resting period was later tabulated from the video recordings. We collected 195 ±
166 12.3 hours of behaviour data/subject during the study period.

167 We measured three behavioural welfare indices: species-typical behaviour diversity
168 (Wemelsfelder et al., 2000), spread of participation index (Plowman, 2003) and aberrant
169 repetitive behaviours or stereotypy (Mason & Latham, 2004) to measure the welfare of Asiatic
170 lions (Goswami et al., 2020). We compared the behavioural welfare indices of all test and control
171 subjects across the baseline and enrichment period. Baseline information for diversity of
172 behaviour repertoire in control and test subjects was collected using Shannon-Weiner diversity
173 index (SWI) (Goswami et al., 2020). We measured enclosure usage patterns of subjects based on
174 the spread of participation index (SPI) (Plowman, 2003)to understand if the enclosures provided
175 enough complexity to meet their welfare requirement (Plowman, 2003; Ross and Shender, 2016).
176 We divided each enclosure (N = 15) into ten zones, i.e., three primary zones (proximal, medial,
177 and distal), which were subdivided into three secondary zones (left, middle, right), and the tenth
178 zone was the area adjacent to the entrance to feeding cubicles (Goswami et al., 2020). We
179 measured the proportion of aberrant repetitive behaviours (ARBs) or stereotypy as an indicator
180 of poor welfare and compared their prevalence between control and test subjects across
181 treatments.

182 ***2.4.2. Physiological measures***

183 We measured faecal corticosterone metabolites of all subjects to contrast the physiological
184 impacts of the enrichment interventions on control and test subjects. Faecal corticosterone is a
185 reliable physiological indicator of stress and compromised welfare in captive felids (Ruskell et

186 al., 2015; Schildkraut, 2016; Vaz et al., 2017; Young et al., 2004). We collected two fresh faecal
187 samples/week from each subject (both control and test) during the entire study period (pre-
188 enrichment and post-enrichment).

189 We collected all fresh faecal samples using the dry sampling approach (Biswas et al., 2019) and
190 stored in -20°C freezer onsite and later transported to the Wildlife Institute of India in dry ice.
191 The samples were stored in the laboratory at -20°C freezer until further processing. To control
192 for effects of moisture and diet we pulverized the frozen samples before lyophilizing (#FD-5,
193 Allied Frost, New Delhi, India) them for 72 hours prior to hormone extraction (Mondol et al.,
194 2020). Subsequently, we sieved lyophilized samples through a 0.5 mm stainless steel mesh to
195 obtain homogenized faecal powder. We thoroughly mixed the dried faecal powder and extracted
196 hormone by pulse-vortexing 0.1 grams of powder in 15 ml of 70% ethanol, followed by
197 centrifugation at 2200 rpm for 20 min (Mondol et al., 2020; Wasser et al., 2010). We collected
198 and stored the hormone extracts in 2 ml cryochill vials (1:15 dilution) and stored in -20°C freezer
199 till further analyses.

200 We used corticosterone EIA kit (#K014, Arbor Assays, MI, USA) for corticosterone metabolite
201 estimation in faecal hormone extracts. Sample extracts were air-dried inside an incubator and
202 resuspended in assay buffer as per required dilutions. Each sample was assayed in duplicate
203 using kit protocol and the optical density was measured at 450 nm using an ELISA plate reader
204 (#GMB-580, Genetix Biotech Asia Pvt. Ltd., New Delhi, India). Hormone metabolite
205 concentration is interpolated using four parametric logistic (4PL) regression function in
206 GraphPad prism software version 5 (GraphPad Software, California, USA). Cross-reactivities of
207 the antibody are listed in Supplementary Table 2.

208 We tested for parallelism and accuracy to validate the corticosterone assay. We used dilutions of
209 pooled extracts from a random combination of male and female samples (N=20) to assess
210 reliable quantification of corticosterone at different concentrations and find optimal dilutions for
211 final assays (at 50% binding). We plotted the relative dose against percent bound hormones for
212 the pools and the standards and generated best-fit curve using 4PL regression, where parallel
213 slopes indicate similar immunoreactivity at different concentrations. For accuracy test, we spiked
214 corticosterone standards with equal volumes of diluted faecal extract of known concentration
215 (dilution level close to 50% bound from parallelism test) and assayed with standards. We plotted
216 the results as regression lines using observed and expected concentrations to show that faecal
217 contaminants were not interfering with assay accuracy at the tested dilution. We calculated inter-
218 and intra- assay coefficients of variation using repeated measures of same-pooled extract.

219 ***2.4.3. Enrichment interventions***

220 Enrichment interventions add cognitive complexity to enclosures and provide animals the
221 opportunity to express species-typical behaviours (Mellen and Shepherdson, 1997; Skibiel et al.,
222 2007). We used three types of enrichment devices: manipulable (Mellen and Shepherdson, 1997;
223 Powell, 1995), sensory (Skibiel et al., 2007), and feed (Powell, 1995; Skibiel et al., 2007) (Table
224 1). Manipulable enrichments included hanging lion-sam balls, burlap bags, wooden perches/
225 platforms, wooden planks etc., whereas the sensory enrichments included olfactory
226 augmentations such as scent trails made from the blood of buffalo, urine of unknown
227 conspecifics, and dung of other prey species (Sambar, *Rusa unicolor*) etc. We also installed
228 sensory enrichment (tactile) by wrapping rough coir rope on the bark of trees inside enclosures to
229 promote auto-grooming and scent-marking behaviours in the subjects. Nutritional enrichment
230 has been shown to garner the highest amount of attention in captive animals and can be useful

231 for addressing neophobia to enrichment devices in shy individuals (Powell, 1995; Resende et al.,
232 2009; Skibiel et al., 2007). We provided a range of nutritional enrichment devices, which
233 included frozen dressed chicken, buffalo tails that were suspended by natural fibre ropes from
234 trees at different parts of the enclosure. While manipulable and sensory enrichment devices were
235 quasi-permanent and required little daily care, nutritional enrichment devices needed to be
236 replenished every day. We installed and replenished enrichment devices between 0600-0700
237 hours at four enclosures every day. Keepers would arrive early and confine the subjects inside
238 retiring cells with food rewards, while the enrichment interventions were placed at designated
239 enclosures. We furnished all enclosure zones with the same combination of manipulable, sensory
240 and nutritional enrichment.
241 To calculate enrichment device preferences, we collated all scans during which Asiatic lions
242 interacted with the enrichment devices. We also recorded the type of behaviours that were
243 performed by the subjects while interacting with the enrichment devices viz., exploration, social
244 play, aggression, foraging and fear (Table 2). We counted the different types of behaviours
245 performed per enrichment device by all subjects.

246 ***2.5. Data analysis***

247 We analysed all data in R v3.6.2 (R Core Team, 2020) with R studio(RStudio, 2020). We used
248 packages "tidyverse" (Wickham et al., 2019), "psych" (Revelle, 2019)and "dplyr(Wickham et
249 al., 2020),"ggpubr"(Kassambara, 2020), "sur"(Harel, 2020) and "ggplot2" (Wickham, 2011) to
250 calculate statistical outputs and create graphical summaries of the findings. We conducted tests
251 for normality and ensured that variances were similar between groups to select appropriate
252 parametrical and non-parametrical statistical analysis. We used unpaired t-test to compare the
253 welfare indices between test and control groups during baseline and post-enrichment

254 interventions. We used paired t-tests, to compare the differences in welfare indices within test
255 and control groups and measured the amount of time spent by test subjects on different types of
256 enrichment devices. Additionally, we calculated the effect size (Cohen, 1992, p. 199)of the
257 enrichment intervention for the test group, which is scale-independent and gives a measure of the
258 magnitude of difference between baseline and post-treatment conditions.

259 **3. Results**

260 ***3.1. Enrichment preferences of study subjects***

261 During 14 days of enrichment intervention, we recorded 1,09,238 scans of test subjects (n=19)
262 using enrichment devices. The test subjects used manipulable enrichment devices for longer
263 durations (56.8%) compared to all other types viz., sensory (12.99%) and feeding (30.20%)
264 (Table 3). It was challenging to measure the usage levels of sensory enrichments accurately and
265 hence these values may have been under-reported in our results. When subjects were released
266 inside the enclosures, they approached the feed-based enrichment devices first followed by
267 manipulable and sensory devices. This trend continued throughout the intervention period. Feed-
268 based enrichment devices were extensively used during the first two hours of the day between
269 0700-0900 hours (Figure 1, Table 4a) and garnered little or no interest after they were depleted.
270 On the other hand, manipulable and sensory enrichment device usage peaked twice daily at 0700
271 and 1600 hours (Figure 1, Table 4a) with continued usage throughout the day.

272 In terms of enrichment directed behaviours, we found that the peak of aggression (6.78%), chase
273 (7.69%), exploratory (6.35%) and forage (7.66%) behaviours in test subjects occurred between
274 0700-0900 hours (Table 4b). The next peak of enrichment-directed behaviours occurred between
275 1300-1700 hours constituting mostly of play (20.66%) and exploratory behaviours (4.11%)
276 (Table 4b). Early in the day (0700-0900 hours), when subjects were actively exploring around

277 the enclosure for enrichment devices, aggressive dominant and displacement behaviours were
278 common but subsided as the day progressed (Figure 1, Table 4b). We found that manipulable
279 devices (11.51%) were more commonly associated with aggressive behaviours compared to
280 food-based enrichment (2.94%) (Table 3). We also observed that positive social behaviours were
281 more commonly associated with food-based enrichment devices (1.16%) rather than the
282 manipulable devices (0.35%) (Table 3). Subjects usually monopolized the manipulable
283 enrichment devices and showed high fidelity towards specific items. The feed-based enrichment
284 devices were associated with the highest proportion of chase behaviour (11.54%), resulting in
285 one animal monopolizing a device and chasing away others (Table 3). Some feed-based
286 enrichment devices were consumed within minutes (e.g. dressed chicken) while others required
287 more processing time (e.g. buffalo tail, frozen blood cubes). Once the feed enrichment devices
288 were completely utilized, the lions concentrated on the manipulable and sensory enrichments and
289 engaged in social and exploratory behaviours throughout the later parts of the day (Table 3,
290 Figure 1). The manipulable enrichment devices were associated with the largest diversity of
291 species-typical behaviours, which included 35.16% play behaviour (Table 3) and were more
292 commonly associated with aggressive interactions (11.51%) compared to all other enrichment
293 types. Aggressive behaviours occurred when enclosure mates tried to steal or use a certain
294 manipulable device from the lion that was playing with it. Since we installed more enrichment
295 devices than subjects in every enclosure, displaced individuals had the opportunity to interact
296 with other devices when displaced by conspecifics. Sensory enrichment devices contributed
297 primarily to exploratory behaviours (11.7%) that led to increased enclosure usage depicted by the
298 lowering of SPI levels (Table 3).

299 However, it is important to point out that the true impacts of different types of enrichment
300 devices cannot be assessed merely through the measuring the amount of time the animal spent
301 with it, but by comparing the welfare indices of test subjects with control and their own baseline
302 status. The results clearly demonstrate that the presence of different types of enrichment devices
303 was instrumental in mitigating monopolization of resources by dominant individuals.

304 ***3.2. Behavioural welfare indices***

305 During pre-enrichment period, behaviour diversity levels of control subjects (M = 0.9, SD = 0.3)
306 were similar to that of the test subjects (M = 0.83, SD = 0.35). After enrichment interventions,
307 the behaviour diversity of control subjects remained unchanged (M = 0.77, SD = 0.35, t(30)=
308 1.11, p = 0.29) while that of the test subjects increased significantly (M = 1.62, SD = 0.18, t(36)
309 = -8.8, p <0.001, Cohen's d = 2.83) (Table 5 Figure 2a).

310 During the baseline, we observed high SPI values in test (M = 0.77, SD = 0.12) and control
311 groups (M = 0.82, SD = 0.09) indicating enclosure zone use bias. However, after enrichment
312 intervention the SPI values decreased significantly indicating, enclosure usage homogeneity (M
313 = 0.46, SD = 0.08) for test subjects (t(36) = 9.37, p <0.0001, Cohen's d = 3.03), while it
314 remained unchanged for control subjects (M = 0.8, SD = 0.13, t(30)= 0.5, p = 0.61) (Table 5,
315 Figure 2b).

316 During baseline, test (M = 12.32, SD = 6.03), and control subjects (M = 12.8, SD = 6) showed
317 similar levels of aberrant repetitive behaviours. After enrichment interventions, we observed a
318 significant decrease in ARBs in test subjects (M = 2.38, SD = 2.46, t (36) = 6.65, p < 0.0001,
319 Cohen's d= 2.15) while the levels remained unchanged for the control group (M = 13.31, SD = 6,
320 t(30) = 0.23, p = 0.81) (Table 5, Figure 2c).

3.3. Faecal corticosterone level

Parallelism and accuracy tests for corticosterone metabolites showed reliable measures from lion faeces across different concentration ranges. Serial dilutions of faecal extracts paralleled the standard curves (Figure 3a). There were no differences between slopes of standard and pooled extract curves for corticosterone ($F(1,10) = 2.06$, $P = 0.182$), and the curves were significantly different in their elevation ($F(1,11) = 66.25$, $P = 0.0001$). Accuracy tests produced slopes of 0.92 at working dilution of 1:60 (Figure 3b), suggesting that faecal extracts did not interfere with their metabolite measurement precisions. Intra-assay coefficient of variation (CV) was 9.8%, whereas inter-assay CV was 8.99% (Supplementary Table 2).

We compared the average faecal corticosterone levels of each subject across baseline and post-enrichment phases. Compared to baseline levels (M = 7.74 μg/gm, SD = 3.2), the faecal corticosterone measures of test subjects decreased significantly (M = 2.46 μg/gm, SD= 0.85, $t(36) = 5.33$, $p<0.0001$, Cohen's d = 2.22), while it remained unchanged from baseline (M = 7.1 μg/gm, SD = 4.08) for control subjects (M= 6.96 μg/gm, SD = 3.93, $t(30) = 0.093$, $p = 0.92$) (Table 5 , Figure 2d). These results confirm that the enrichment interventions led to significant improvement in behavioural and physiological welfare indices of test subjects as compared to the control group during pre-enrichment period.

4. Discussion

Animal welfare is not limited to the prevention of cruelty and amelioration of symptoms of stress, but also accords the opportunity to express species-typical behaviour patterns (Broom, 2011). The National Zoo Policy of India (MoEF,India, 1998) espouses animal welfare with captive animal management, yet welfare-centric management protocols remain divorced from husbandry practices across several zoos. Although few and far between, welfare research from

344 Indian zoos report a prevalence of stereotypic behaviours (Goswami et al., 2020; Mallapur et al.,
345 2007; Sanyal, 1892; Vaz et al., 2017) and elevated faecal corticosterone levels (Vaz et al., 2017)
346 across several captive felids, and prescribe specie-appropriate enclosure design and enrichment
347 interventions as remedial measures (Goswami et al., 2020; Mallapur et al., 2007; Vaz et al.,
348 2017). Enrichment interventions have been shown to improve welfare conditions in several
349 captive felids including African lions (Mellen and Shepherdson, 1997; Powell, 1995; Skibiel et
350 al., 2007), yet enrichment protocols are seldom incorporated in the daily management of captive
351 Asiatic lions at Indian zoos. The Asiatic lion conservation breeding programme has successfully
352 maintained a viable captive population insulated from stochastic ecological events. Most
353 research pertaining to captive Asiatic lions investigate their genetic and demographic status
354 (Bagatharia et al., 2013; Pastorino et al., 2017) with only a handful mentioning their welfare
355 (Goswami et al., 2020; Mallapur et al., 2002; Pastorino et al., 2017; Vaz et al., 2017). Our study
356 is the first to assess the effects of enrichment protocols on the welfare of captive Asiatic lions.
357 Welfare encompasses both internal and external conditions affecting an animal and hence must
358 be measured using a multifarious approach. As highlighted by Miller et al. (2020) the focus on
359 negative welfare indicators has led to zoos adhering to minimum husbandry guidelines that
360 attempt to suppress symptoms of poor welfare rather than trying to improve existing conditions.
361 In this study, we measured two traditional negative welfare parameters like ARB (Mason and
362 Latham, 2004) and faecal corticosterone levels (Schildkraut, 2016; Vaz et al., 2017) along with
363 two positive welfare indicators (Miller et al., 2020) viz., spread of participation index (SPI)
364 (Cabana et al., 2018; Powell, 1995) and behaviour diversity (Pastorino et al., 2017). Previous
365 studies on captive African lions that establish the positive effects of enrichment interventions
366 have primarily relied on behavioural welfare indices (Martínez-Macipe et al., 2015; Ncube and

367 Ndagurwa, 2010; Powell, 1995; Regaiolli et al., 2019; Van Metter et al., 2008). Our study is the
368 first to showcase both the behavioural and physiological impacts of enrichment interventions in
369 captive Asiatic lions housed in a conservation breeding programme. The welfare evaluation
370 framework used in this study can be applied universally (for other species) to measure the
371 impacts of goal-oriented enrichment interventions.
372 While previous studies have tested the welfare impacts of food (Powell, 1995; Van Metter et al.,
373 2008), manipulable (Powell, 1995), sensory (Martínez-Macipe et al., 2015; Regaiolli et al., 2019)
374 and social stimulation (Leonard, 2008; Ncube and Ndagurwa, 2010) individually. This is the first
375 control trial study to test the efficacy of a combined enrichment strategy on the welfare of Asiatic
376 lions. This has allowed us to understand the temporal usage pattern of enrichment devices in
377 Asiatic lions, which can prove useful for tailoring enrichment strategies. For example, individual
378 test subjects showed high fidelity towards manipulable enrichment devices, which were also
379 associated with the highest amount of conspecific-directed aggression. Therefore, to reduce
380 stress and aggressive behaviours we recommend providing more manipulable devices than the
381 number of animals in an enclosure. Upon release, subjects rushed to the feeding enrichment
382 devices, therefore to prevent aggression and injury, it is important to spread such devices far
383 apart, which will reduce chances of monopolization and food-based aggression. While sensory
384 enrichment devices are designed to encourage exploratory behaviours in lions, the feed-based
385 enrichment devices create a positive association for these novel objects and reduce neophobia.
386 Finally, we found that manipulable enrichment devices allow subjects to interact and exert
387 control over the captive environment while engaging in species-typical behaviours. Our
388 combined enrichment strategy brought novelty in a captive environment and presented animals
389 with the choice to express species-typical behaviour patterns. All enrichment devices used in this

390 study can be locally sourced or fabricated with minimal effort and require less than thirty
391 minutes to install or replenish with a team of three people.
392 Post-enrichment intervention, we recorded a significant improvement in the welfare indices of
393 test subjects compared to the control subjects. Subjects that would normally stay near the retiring
394 cells and remain inactive all day started exploring and utilizing different parts of the enclosure
395 and followed by a significant increase in behaviour diversity levels. Faecal corticosterone levels
396 of test subjects decreased significantly from the baseline, while it remained unchanged for
397 control subjects, showing the inter-linkages between behavioural and physiological indices of
398 welfare. Our findings agree with existing research that associate enrichment interventions with
399 increased behavioural diversity (Rabin, 2003; Wemelsfelder et al., 2000), lowered SPI (Nogueira
400 et al., 2004; Rose and Robert, 2013; Traylor-Holzer and Fritz, 1985), reduction of ARB (Clark
401 and Melfi, 2012; Mallapur et al., 2007; Vaz et al., 2017), and reduction of stress levels
402 (Hutchinson et al., 2012; Marcon et al., 2018; Mitra and Sapolsky, 2009; Nazar and Marin,
403 2011). However, it is important to point out that due to logistic constraints (sample size, time
404 etc.) we did not address the variable responses of subjects with different personality types on
405 enrichment interventions. Earlier work on captive Asiatic lions indicated that lions with different
406 personality traits are likely to react differently to novel enrichment devices (Goswami et al.,
407 2020). We suggest that future studies should focus on addressing impacts of such individual
408 variations on differential responses and enrichment preferences with larger sample size. We
409 studied the largest captive population of Asiatic lions, but the methodologies of this study can be
410 scaled for smaller zoos housing fewer animals. Although we used at least three types of
411 enrichment devices per intervention at multiple enclosure zones for experimental uniformity, at a
412 smaller scale, zoo managers can try one enrichment device type per enclosure and rotate them

413 with a new one every week. Our results conclusively show that enrichment interventions can
414 lead to better behavioural and physiological welfare for Asiatic lions. We have delineated a
415 practical but holistic approach to enrichment interventions that can be implemented and tested
416 with little effort and manpower. With this study, we hope to empower and enable zoo managers
417 and regulatory agencies to mandate the incorporation of enrichment interventions into daily
418 husbandry regimen and proactively improve the welfare status of the animals under their care.
419 We sincerely hope that the results of this study contribute to the improvement in animal welfare
420 practices across ex-situ institutions at Indian zoos and conservation breeding programs.

421 **5. Conclusion**

422 The success of conservation breeding program depends on repatriating endangered animals to
423 reclaimed habitats, hence housing the animals in appropriate welfare conditions while
424 persevering behaviour diversity is of utmost importance. The Asiatic lion conservation breeding
425 program has successfully maintained a genetically diverse stock of the species in captivity that
426 has proliferated to an appreciable number. With the incorporation of welfare-centric management
427 practices, the Asiatic lions housed in the program can be ideal representatives of the species and
428 great candidates for future reintroduction programmes. Until that day of repatriation, we should
429 strive to create a cognitively enriching environment that preserves the species-specific traits of
430 the Asiatic lions. Like the endangered Asiatic lion, the Indian ex-situ institutions run
431 conservation breeding programmes for several endemic species. So far, the welfare status of
432 animals housed under conservation breeding programmes has received little research attention.
433 We hope this study encourages zoo managers and regulators to incorporate enrichment
434 interventions with animal management protocols.

Ethics statement

This study was conducted in accordance with CZA norms on the welfare of animals housed in conservation breeding centres. We did not conduct any invasive sampling procedures on the subjects for experimental setup and data collection. All necessary precautions were taken to ensure that enrichment interventions were integrated with the daily husbandry regimens of the test subjects.

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636 **Figure legends**

637 **Figure 1:** Temporal pattern of enrichment device use and behavioural patterns by Asiatic lions
638 on (a) Feeding, (b) Manipulable, and (c) Sensory enrichment devices. Primary Y- axis represents
639 the observation period from 0700-1800 hours. The primary x-axis represents the scan counts
640 (log10 scale) of specific behaviours on each type of enrichment device. Hourly counts of
641 enrichment-directed behaviours are represented as colour bars on the horizontal axis.

642 **Figure 2:** Comparison of the welfare indices between test and control groups across pre- and
643 post-enrichment conditions. The four different welfare indices used in this study are (a)
644 Behaviour diversity; (b) Spread of participation index; (c) Aberrant repetitive behaviours (ARB)
645 and (d) Faecal corticosterone levels.

646 **Figure 3:** Standardization of lion faecal corticosterone through (a) parallelism and (b) accuracy
647 tests. The graphs show accurate measures of corticosterone from captive lion faecal samples.

648 **Supplementary Figure 1:** Schematic representation of enclosures at the SZG conservation
649 breeding center for Asiatic lions.

Table 1: Description of enrichment devices used in this study at enclosures

Sl. no	Enrichment device	Enrichment type	Description
1	Platform	Manipulable	A log platform made to enable subjects to access vantage points within the enclosure
2	Sam ball	Manipulable	A hard-plastic ball with no holes or perforations
3	Log branches	Manipulable	Tree logs sections with bark suspended from trees
4	Lion bite ball	Manipulable	A hard-plastic ball with a rattle inside suspended by a bungee rope with canvas sheet that acts as a bite
5	Buffalo shank	Feeding	Buffalo shank suspended from a zipline
6	Chicken carcass	Feeding	Frozen Chicken carcass suspended from a tree by natural fibers
7	Buffalo tail	Feeding	Skinned buffalo tail suspended from tree branches
8	Buffalo blood	Feeding	Frozen buffalo blood cubes, provided on a clean concrete platform
9	Star anise oil mixed with garlic	Sensory	Sprayed on tree trunks or used to create scent trails to other enrichment devices
10	Prey dung	Sensory	Sambar (*Rusa unicolor*) dung smeared on tree trunks or mixed with enclosure soil at certain zones
11	Conspecific urine	Sensory	Earth drenched in the urine conspecifics from nearby enclosures smeared on tree trunks, or mixed with enclosure substrate at certain zones

Table 1: Behaviours observed that are associated with different enrichment devices

Sl. no	Behaviour	Description	Category
1	Aggressive Growl	Baring teeth with short vocalizations directed at conspecifics or humans, while interacting with enrichment	Aggression
2	Charge	Run up to a conspecific at a rapid short charge as a result of being challenged while suing an enrichment device	Aggression
3	Strike	Hit conspecific with one paw, while defending an enrichment device	Aggression
4	Bite	Bite conspecific, while asserting control over an enrichment device	Aggression
5	Allogrooming	Lick conspecific, while interacting with enrichment device	Social
6	Nuzzling	Rub noses with conspecifics with soft grunts, and inviting them to play with enrichment device	Social
7	Roll	Rolling in the dirt with conspecific while interacting with a enrichment device	Social
8	Rub	Rub body or neck against the enrichment device	Explore
9	Sniffing	Raise head and sniff near a sensory enrichment	Explore
10	Flehmen	Bare teeth and sniff the air near an enrichment device	Explore
11	Scent mark	Spray urine or scratch the tree next to an enrichment device	Explore
12	Digging	Dig near an enrichment device	Explore
13	Scratching	Scratch the trees where enrichment devices are installed	Explore
14	Chase	Chase away conspecifics trying to use or steal enrichment device successfully or unsuccessfully. Does not result in an overtly aggressive interaction	Chase
15	Run	Run around the enclosure with the enrichment device in mouth. May or May not be chased by conspecific	Play
16	Stalk	Stalk an enrichment device	Play
17	Play	Kick around an enrichment device	Play
18	Drag	Drag enrichment device along the enclosure	Play
19	Bite	Bite enrichment device	Play
20	Eat	Eat a food based or sensory enrichment device	Forage
21	Lick	Lick enrichment devices intently	Forage
22	Neophobia	Approach enrichment device slowly and run away	Fear
23	Fearful growl	Growling at enrichment device, ears back and cowering	Fear
24	Circling	Warily circling around the enrichment device	Fear

Table 1: Variations in behavioural performances in different enrichment devices used in this study.

Behaviour category	**Feed enrichment (%)**	**Manipulable enrichment (%)**	**Sensory enrichment (%)**
Aggression	2.94	11.51	0
Chase	11.54	1.89	0
Explore	3.05	5.95	11.77
Fear	0	1.96	0
Forage	11.48	0	0
Play	0	35.16	1.21
Social	1.16	0.35	0
Total	30.20	56.8	12.99

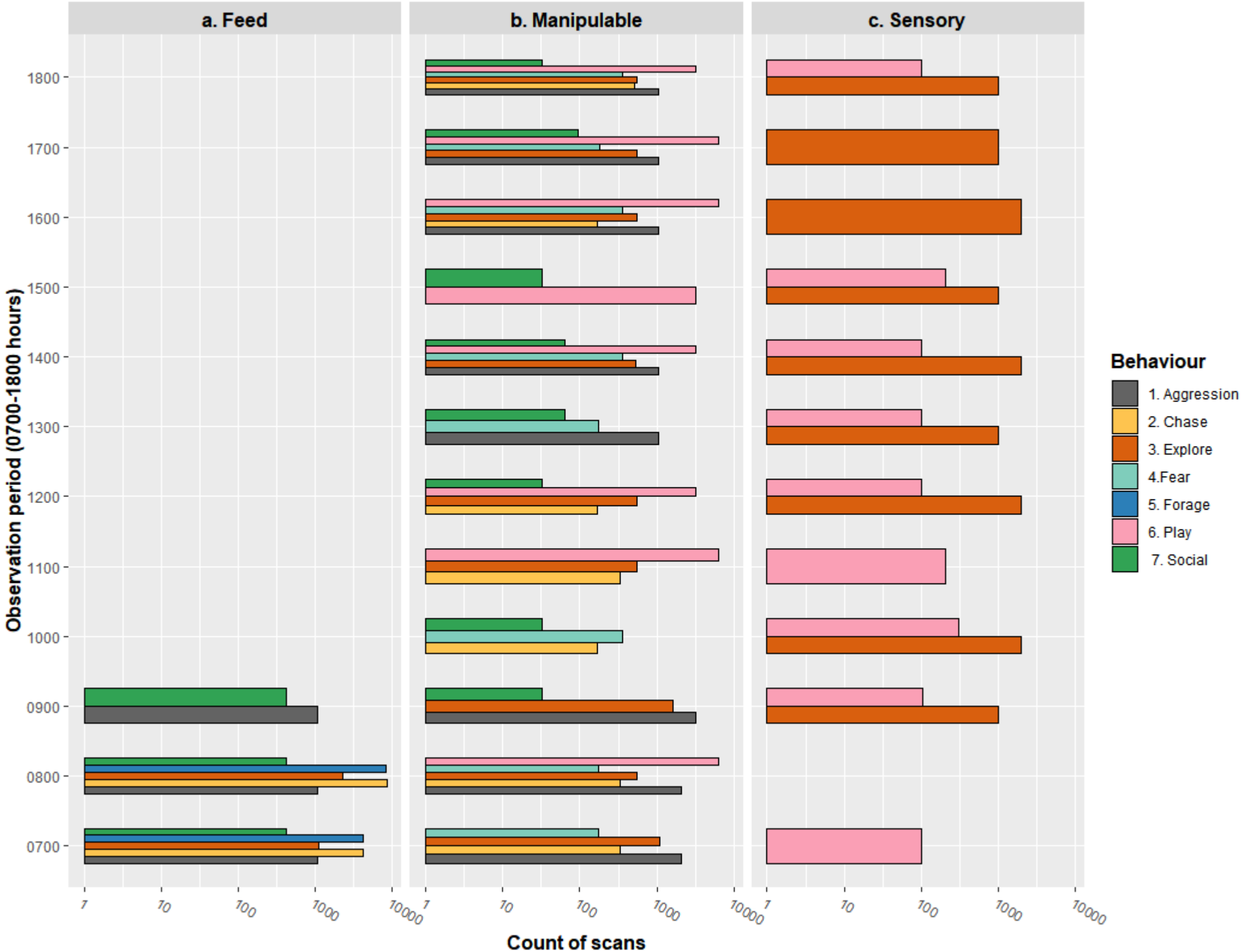
Table 4: Temporal patterns of enrichment usage and behavioural categories in the test subjects (n=35) during enrichment intervention experiments. The upper part of the table presents the enrichment use pattern, whereas the lower part of the table shows behaviour categories.

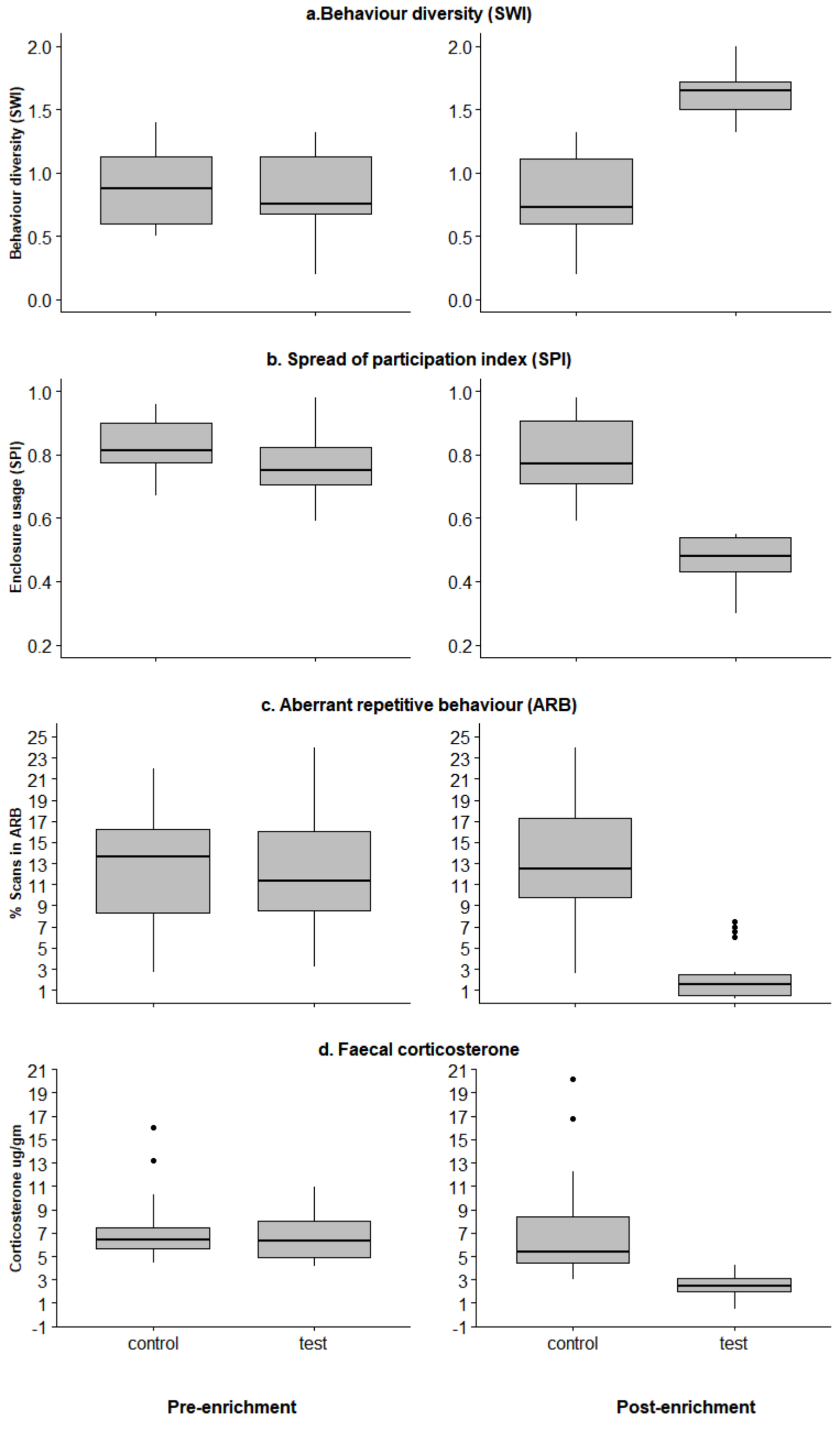
Enrichment use pattern at temporal scale (%)							
Enrichment	**0700-0900**	**0900-1100**	**1100-1300**	**1300-1500**	**1500-1700**	**1700-1900**	**Total**
Feed	22.13	8.06	0	0	0	0	30.2
Manipulable	11.269	6.94	7.02	23.44	5.66	2.44	56.8
Sensory	1.9	2.0	3.18	0.99	3.81	1.09	12.99
Behaviour categories at temporal scale (%)							
Behaviours	**0700-0900**	**0900-1100**	**1100-1300**	**1300-1500**	**1500-1700**	**1700-1900**	**Total**
Aggression	6.78	1.91	1.97	0.95	1.91	1	14.57
Chase	7.69	4.31	0.92	0	0.15	0.30	13.40
Explore	6.35	2.8	3.71	2.39	4.11	1.4	20.78
Fear	0	0.49	0.16	0.48	0.16	0.65	1.96
Forage	7.66	3.83	0	0	0	0	11.48
Play	5.95	3.11	3.39	20.6	3.11	0.18	36.29
Social	0.86	0.53	0.08	0	0	0.03	1.52

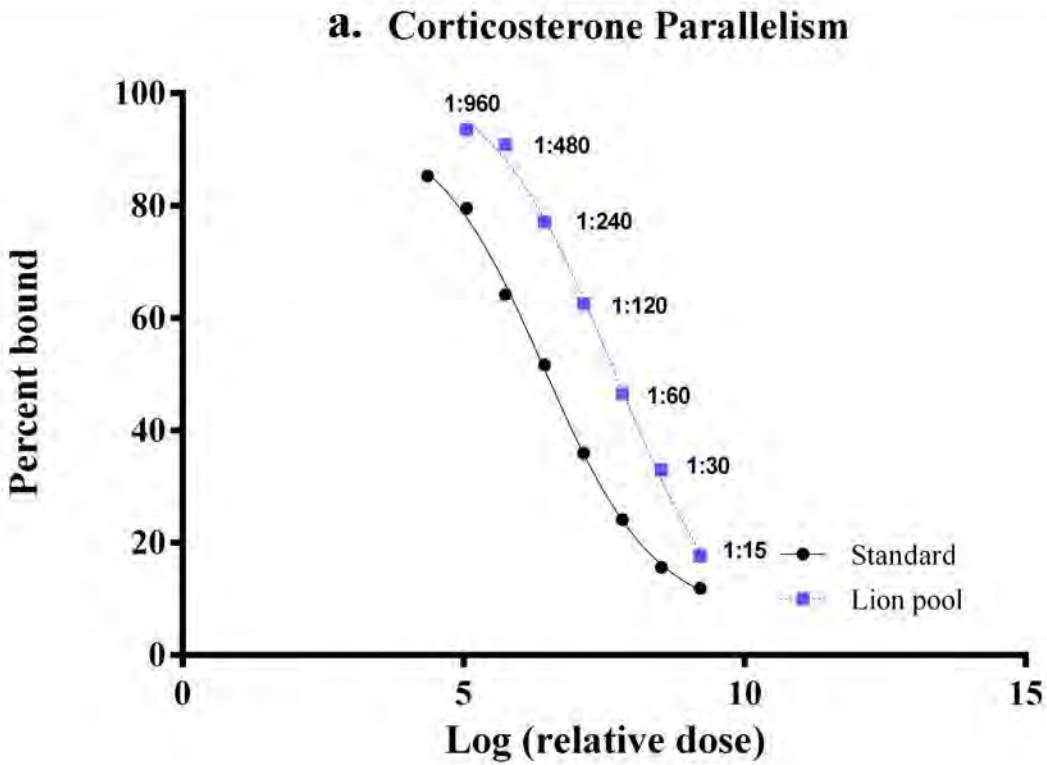
Table 5: Summary statistics of welfare indices within control and test groups

Welfare indices	Pre-enrichment	Post-enrichment	t-value*	Significance (p-value)	Effect size (Cohen's d)
Control group (N=16)					
Behaviour diversity (SWI)	0.9±0.31	0.77±0.35	1.11	0.29	0.39
Spread of participation index (SPI)	0.82±0.09	0.8±0.13	0.51	0.61	0.17
Aberrant repetitive behaviours (ARB)	12.81±6	13.31±6.07	0.23	0.81	0.08
Faecal corticosterone measure	7.1±4.08	6.96±3.93	0.09	0.92	0.03
Test group (N = 19)					
Behaviour diversity (SWI)	0.83±0.35	1.62±0.18	-8.8	0.0001	2.83
Spread of participation index (SPI)	0.77±0.12	0.46±0.08	9.37	0.0001	3.03
Aberrant repetitive behaviours (ARB)	12.32±6.03	2.38±2.46	6.65	0.0001	2.15
Faecal corticosterone measure	7.74±3.25	2.46±0.85	6.85	0.0001	2.22

* For control group t(30) and test group t(36).


a. Feed
b. Manipulable
c. Sensory
Observation period (0700-1800 hours)
1800
1700
1600
1500
1400
1300
1200
1100
1000
0900
0800
0700
1
10
100
1000
10000
Count of scans
Behaviour
1. Aggression
2. Chase
3. Explore
4.Fear
5. Forage
6. Play
7. Social


a.Behaviour diversity (SWI)
Behaviour diversity (SWI)
2.0
1.5
1.0
0.5
0.0
b. Spread of participation index (SPI)
Enclosure usage (SPI)
1.0
0.8
0.6
0.4
0.2
c. Aberrant repetitive behaviour (ARB)
% Scans in ARB
25
23
21
19
17
15
13
11
9
7
5
3
1
d. Faecal corticosterone
Corticosterone ug/gm
21
19
17
15
13
11
9
7
5
3
1
-1
control
test
Pre-enrichment
Post-enrichment


a. Corticosterone Parallelism
Percent bound
Log (relative dose)
1:960
1:480
1:240
1:120
1:60
1:30
1:15
Standard
Lion pool
0
20
40
60
80
100
0
5
10
15

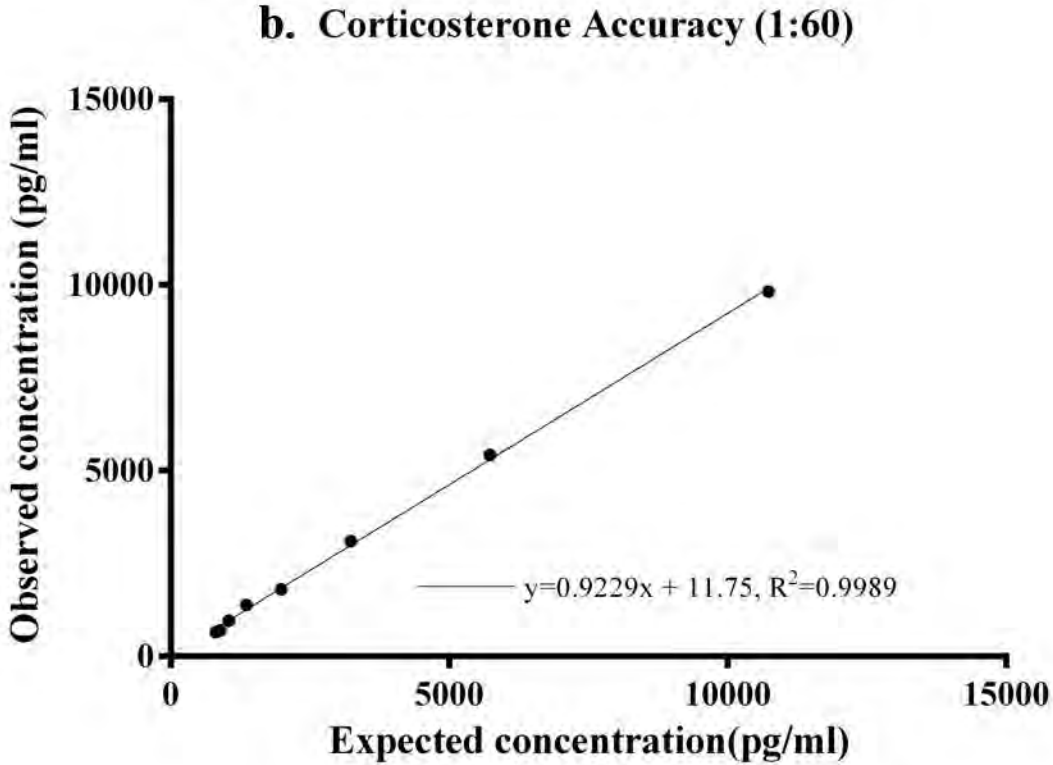
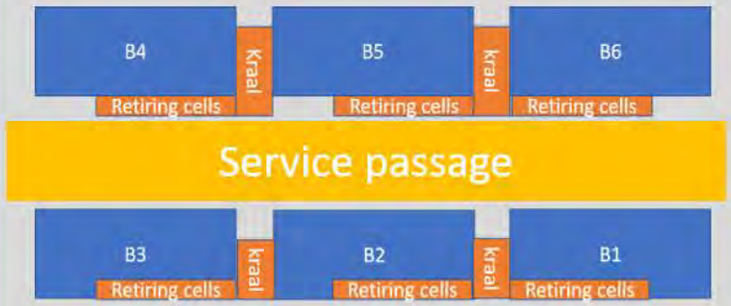

b. Corticosterone Accuracy (1:60)
Observed concentration (pg/ml)
Expected concentration(pg/ml)
y=0.9229x + 11.75, R2=0.9989
0
5000
10000
15000
0
5000
10000
15000

Table 1: Details of subjects included in the study, viz., house name, sex (M=Male, F=Female), origin (C=Captive, W=Wild), sex ratio for housing, enclosure size, space per animal, age in days, and group.

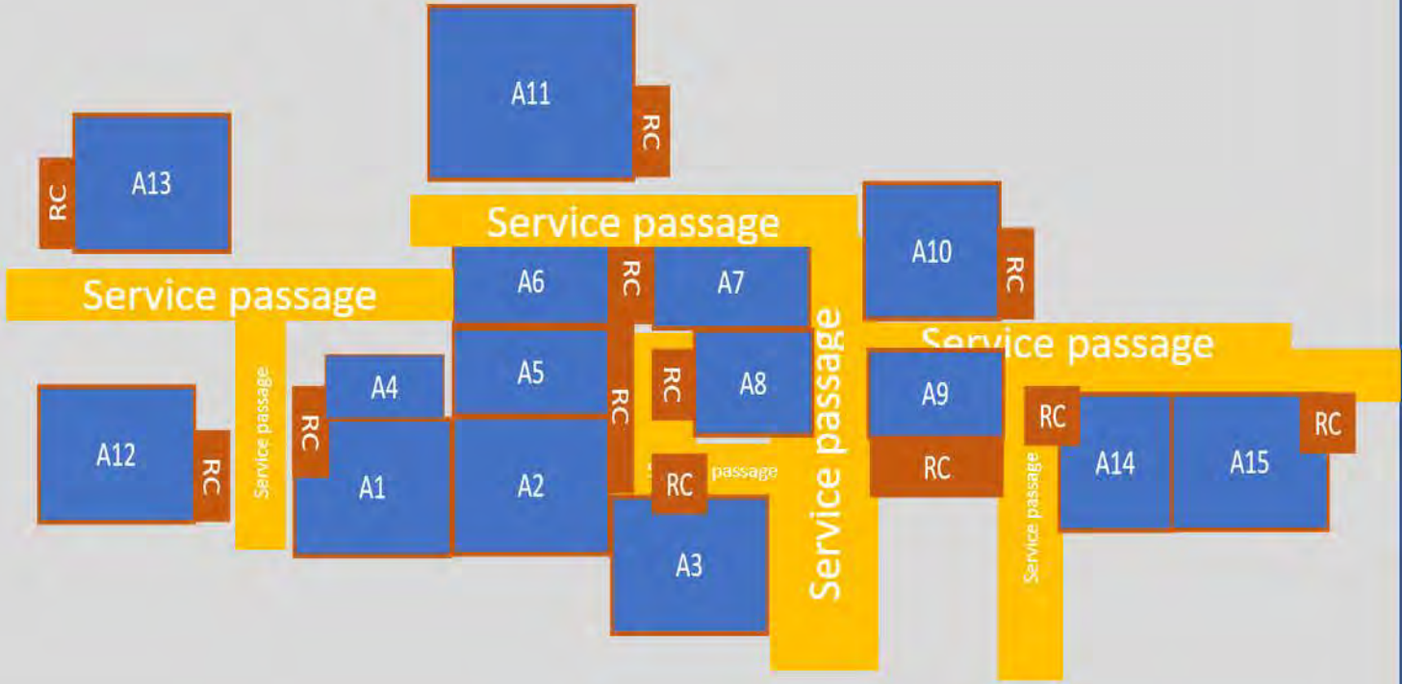
Sl no	Name	Sex	Origin	Sex ratio (M:F)	Enclosure size (m^2)	Space/animal (m^2)	Age (in days)	Group
1	A1	M	W	1:1	1300	650	3414.00	Control
2	Aftab	M	W	1:1	1200	600	3500.00	Control
3	Amal	M	C	1:2	1576	788	2214.74	Test
4	Ambica	F	C	0:2	1123	561.5	3984.56	Test
5	Amiya	F	C	0:2	1123	561.5	2037.56	Test
6	Ani	F	W	1:1	1600	800	1946.00	Test
7	Bahadur	M	C	1:3	1600	533.33	653.33	Test
8	AmrapurI	F	W	1:3	1600	533.33	800.33	Test
9	AmrapurII	F	W	1:3	1600	533.3	800.33	Test
10	Dhari	M	W	1:3	1100	366.6	619.50	Test
11	Dheer	M	W	2:0	1600	800	5389.08	Control
12	Gina	F	C	1:1	1100	550	1316.90	Control
13	Girm	M	W	1:1	1123	562.5	3337.92	Control
14	Hemal	M	W	1:2	1700	566.6	2029.00	Control
15	Hemali	F	W	1:2	1271	635.5	2149.00	Control
16	Jenifer	F	C	1:1	1700	850	1491.33	Test
17	Jesal	M	W	1:2	1300	433.3	5722.18	Test
18	Maheswari	F	C	1:2	1300	433.3	3411.26	Test
19	Mariyam	F	C	1:2	1300	433.3	3423.35	Test
20	Maytri	F	C	1:1	1123	562.5	3072.90	Control
21	Nagraj	M	W	1:2	6542	2180	3227.00	Control
22	Patvad	F	W	1:1	1200	600	3729.00	Control
23	Patvadm	M	W	1:1	1271	635.5	1537.00	Test
24	Radha	F	C	1:2	1576	525.3	1291.30	Test
25	Rani	F	C	1:2	1576	525.3	1291.30	Test
26	Ranita	F	C	1:2	6542	2180	2119.00	Control
27	Ranshi	F	W	1:2	6542	2180	5109.00	Control
28	Rudi	F	W	1:1	1600	800	4700.00	Control
29	Subhi	F	C	1:1	1700	850	2240.33	Test
30	Sujan	F	W	1:1	1300	650	2790.80	Control
31	Taukir	M	C	1:1	1303	651.5	2448.00	Control
32	Teeta	F	C	1:2	1700	566.6	2166.93	Test
33	Tejaswini	F	C	1:2	1700	566.6	4320.93	Test
34	Trakuda	M	W	1:2	1700	566.6	3956.93	Test
35	Veer	M	W	2:0	1600	800	5387.30	Control

Supplementary Table 2: Details of the lion faecal corticosterone assay conducted in this study.

Hormone	**Assay method**	**Dilution**	**Slope (R^2)**	**Inter-assay CV**	**Intra-assay CV**	**Cross-reactivity**
Corticosterone	EIA	1:60	0.92 (0.99)	9.0	9.8	100% with corticosterone, 12.3% with Desoxycorticosterone, 2.3% with Tetrahydrocorticosterone and <1% with Aldosterone, Cortisol, Progesterone, Dexamethasone, Corticosterone-21-Hemisuccinate, Cortisone and Estradiol



Complex B of Asiatic lion CBC



Complex A of Asiatic lion CBC