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Original Article

Effects of Complex Semi-Natural Cage System on Rat Welfare using Behavior, Fecal Glucocorticoid Metabolites and Selected Organ Weights as Indicators

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ABSTRACT

Introduction: Limited space often presents a significant challenge in laboratory animal housing systems, as it restricts the animals' capacity to exhibit species-specific behaviors due to enclosures and structural designs that differ from their natural habitats. The present study aimed to develop a complex caging system (semi-natural cages) equipped with different enrichment items to assess the welfare of rats housed in these cages by observing home-cage behaviors, measuring fecal glucocorticoid metabolites, body weight, and certain organ weights.

Materials and methods: Twenty-four, twelve-week-old female Sprague-Dawley rats with a mean weight of 239 ± 19 grams were randomly allocated to three semi-natural and three standard cages (four rats in each cage) and studied weekly for six weeks. Behavioral data were collected from four rats housed in each of the two cage types for five weeks via data-stamped video footage, which was randomly scored using scanning and focal analyses. Fecal glucocorticoid metabolite (FCM) concentrations were measured for six weeks using the Invitrogen progesterone competitive ELISA Kit, adapted for measuring 5a-pregnan-3b,11b,21-triol-20-one competitive enzyme immunoassay. Rats were weighed weekly, and the weights of the brain, thymus, spleen, and adrenal glands were measured at the end of the study.

Results: Rats in semi-natural enriched cages were more active than in standard cages. Significantly higher counts of enrichment-directed (U = 617) and non-intake (U = 1,908.5) behaviors were recorded in semi-natural cages compared to the standard cages. The counts of social interaction behaviors were significantly higher in standard cages (U = 2,255) than in semi-natural cages. No significant differences in body weight and organ weights were observed among the rats in the two cage types. Average FCM concentrations indicated periodic fluctuations and an overall upward trend over time in both housing systems. There was no significant difference between the mean FCM concentrations of rats housed in the two cage types.

Conclusion: The current findings supported the use of a semi-natural cage-housing system in rats.

1. Introduction

Animal housing is one of the major factors that impact animal studies in laboratory animal facilities[1]. In many laboratory animal facilities, the enclosures for animals are designed in ways that do not accurately represent their natural habitats[2,3]. Space is the primary constraint in designing laboratory animal housing[4], since larger facilities increase operational costs and most facilities are housed in existing buildings not originally intended for animal care[5]. Laboratory rats exemplify a species greatly affected by housing conditions, as they are kept in cages that differ structurally from their natural habitat. Despite their utilization in investigations, rats continue to be nocturnal

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animals characterized by a well-defined biological rhythm clock[6]. In natural habitats, rats live in burrows[7] in social groups of up to hundreds of animals, with well-established dominance hierarchies[8]. Rats exhibit behaviors such as nest building, climbing, foraging, exploration, and hiding in their natural habitats. Housing systems that facilitate social behavior, burrowing, climbing, foraging, and nest building have been recommended in laboratory environments[1,9,10]. Studies suggested that allowing animals to display their natural species-specific behaviors in a natural setting can enhance their resilience to stress[11].

Generally, there are two types of rat housing systems, including research housing and domestic housing[12]. Besides serving as tools for scientific experiments, some laboratory rat strains, such as Sprague-Dawley and Long-Evans rats, are often kept as companion animals and readily adapt to these domestic environments[10]. In these domestic environments, the rats are typically housed in complex caging systems furnished with different types of enrichment items such as burrowing substrate, climbing structures, and foraging and nest building material, and exhibit behaviors such as burrowing, foraging, nest building, and exploration, as is normally observed in their natural habitat[1]. In contrast, research rats are housed in simple polycarbonate shoebox-shaped cages with or without limited environmental enrichment items, such as one or two polyvinyl chloride (PVC) tunnels and nesting material, and the environments are not as complex as those in house pet cages[1]. Therefore, it is not surprising that some studies report signs of compromised welfare.

Prolonged exposure to housing environments that deprive laboratory animals of natural behaviors can compromise animal welfare[13]. Animals in poor welfare conditions exhibit altered psychological and physiological issues and are likely to influence the results and data of investigations[14]. These physiological and behavioral changes, called the stress response, enable animals to cope with stressors in their natural environments[15]. During the stress response, changes in endogenous mediators such as corticosterone, catecholamines, and neuropeptides within the body result in physiological and behavioral outcomes depending on the magnitude and duration of the stressor[16]. Rodents respond to stressors by releasing corticosterone via the hypothalamic-pituitary-adrenal (HPA) axis, and hormone levels can reflect their well-being[17]. The weights of internal organs, such as the thymus and adrenal glands, are useful indicators of animal welfare[15,18]. Prolonged exposure to stressful conditions or unpleasant environments leads to adrenal hypertrophy and an increase in adrenal gland weight[19], while thymus gland weight reduces[20]. In terms of behavior, the animals tend to respond to stressors by escaping and retreating into their burrows and shelters[1]. Measurements of behavioral and physiological stress responses are collectively employed to indicate the animal's well-being[14]. The present study aimed to design semi-natural cages furnished with different types of enrichment items and to monitor the welfare of rats housed in these cages using home-cage behaviors, fecal glucocorticoid metabolites (FCM) concentration, body weights, and selected organ weights.

2. Materials and methods

2.1. Ethical approval

The present study was approved by the University of Pretoria Faculty of Veterinary Science Research Ethics (REC), Pretoria, South Africa, and University of Pretoria Animal Ethics (AEC) committees (Protocol Number REC022-20), Pretoria, South Africa.

2.2. Animals

Twenty-four 12-week-old female Sprague-Dawley rats (239 ± 19 g) were used for the present investigation. The number of animals was determined utilizing data from a study conducted by Abou-Ismail and Mahboub[21], in conjunction with G-power calculations[22]. The rats were sourced from South African vaccine producers (SAVP), Johannesburg, South Africa, and housed at the Onderstepoort Veterinary Animal Research Unit (OVARU), Pretoria, South Africa, under controlled temperature (22 ± 2°C), humidity (45 ± 20%), and light/dark (12hr/12hr) cycle[23]. During the 14-day acclimation period, the rats were pair-housed in 12 cages, similar to those used by the breeders (1500U Eurostandard type IV S, Tecniplast, Italy), with wood shavings as bedding. Throughout the study period, all rats had ad libitum access to Epol rodent cubes (Epol®; Epol Pretoria, South Africa) and reverse osmosis water. The rats were checked twice daily, and their cages were cleaned, and fresh bedding and enrichment items were provided once per week.

2.3. Experimental treatments

After the acclimation period, the rats were randomly allocated to six cages, three semi-natural and three standard, each housing four rats (Figure 1), and were housed for six consecutive weeks. Each cage served as an experimental unit.

The standard cages were 1290D Eurostandard type III cages made of polycarbonate and measuring 425 × 266 × 155 mm (L × W × H), manufactured by Tecniplast (Décines-Charpieu, France; Figure 1A). Each cage contained wood shavings as bedding, one PCV pipe, and two pieces of egg tray paper for enrichment.

The semi-natural cages were custom-modified rabbit cages at OVARU Pretoria, South Africa, made of galvanized wire bars, measuring 640 × 450 × 450 mm (L × W × H). The semi-natural cage was equipped with autoclaved burrowing substrate (black earth and compost), nesting material (Figure 1B), two polyvinyl chloride pipes, one nest box, two climbing structures, and gnawing objects (Figure 1C). These items likely allowed rodents to perform natural behaviors such as burrowing, foraging, nest building, climbing, and exploring habitats. Only cage material and enrichment items that were cheap and readily available from a local pet shop (Petmasters, Pretoria, South Africa) were purchased. All items were autoclaved before placing the animals in the cages. Items such as bedding and egg trays were used only once. The PVC pipe and cages were cleaned and autoclaved for reuse.

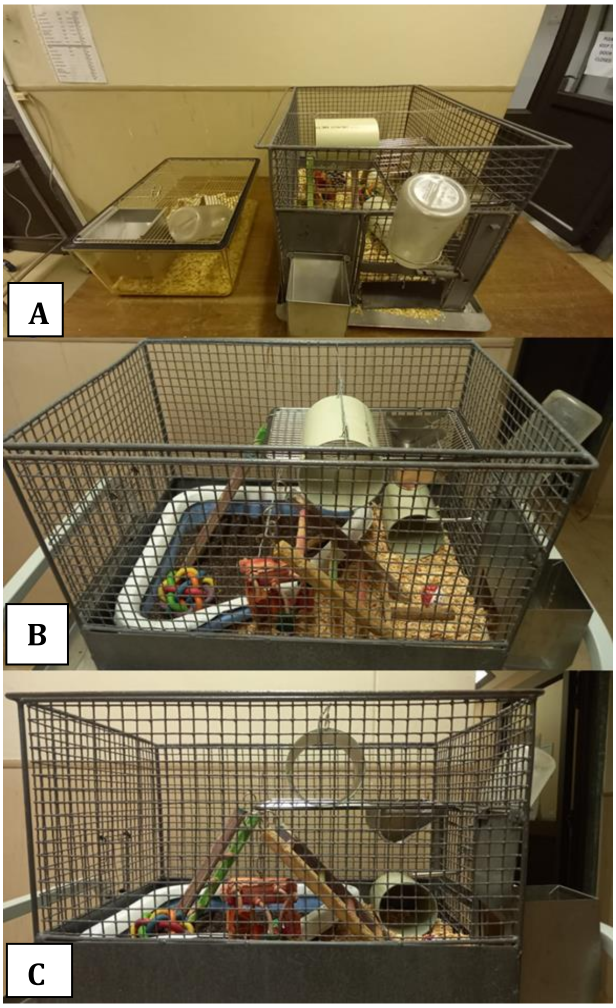

Figure 1. The standard and semi-natural cages for housing the 12-week-old female rats, and enrichment items. **A**: Size differences between the standard cage (on the left) and the semi-natural cage (on the right). **B** and **C**: Different enrichment items provided for the semi-natural cages, namely, burrowing substrate, nesting material, two polyvinyl chloride pipes, a climbing structure, a horizontal rope across the cage, and gnawing objects.

2.4. Data collection

2.4.1. Home cage observations

The rats were recorded for five weeks in their home cages using a digital video recorder (Hikvision DVR, Model: DS-9016HFI-S, Hikvision corporate solutions, Alberton, South Africa, and Samsung camera, SHC721AP model (Samsung Electronics, Johannesburg, South Africa) to gather information about the behavior they exhibited in the two types of cages. Eight sets of video footage (observation sessions) of two hours' length were selected for behavior scoring using the scan and focal sampling methods (25). The two-hour-long observation sessions were randomly selected from the continuous 24-hour video records at every one-hour interval (two-hour-long video clips after every one-hour interval). Evaluations were conducted weekly by the same person throughout the study. For both sampling methods, the observed behaviors were manually recorded onto the respective forms.

2.4.1.1. Scan sampling

The video clips of cages were scanned according to a method described by Martin and Bateson[24]. Briefly, each video clip of each cage was observed for approximately 10 seconds at 10-minute intervals over the 2-hour session. Animals were categorized as active when exhibiting locomotion, self-maintenance, feeding, or exploratory behaviors, and as inactive when stationary in a lying or sitting posture. Each observation session lasted two hours and yielded 12 scans, for a total of 96 scans per cage per day (eight observation sessions) or per observation week.

2.4.1.2. Focal sampling

The video clips of cages were sampled every 15 minutes for five minutes to record counts of behaviors exhibited by the animals. Each cage was observed 8 times per observation session (2 hours), and observed behavior counts were recorded manually on the forms. A selection of behaviors of interest is shown in the ethogram in Table 1.

Table 1. Ethogram of scored behaviors and their definitions in 12-week-old rats

Behaviour	Description
Active behaviour	Rats engage in behaviors such as feeding and drinking, non-intake maintenance, movement activities, exploratory and enrichment-directed behavior, and social interactions.
Inactive behaviour	Rats are asleep or awake but inactive.
Intake behaviour	Rat feeding, eat faeces, or anything in the cage, such as bedding material, and drink water
Non-intake and exploratory behavior	Rats self-grooming, and standing upright on two hind legs (stretching and yawning), moving and/or climbing the cage enclosure, and sniffing.
Enrichment-directed behavior	Rat climbing and manipulating the enrichment objects and bedding material, self-grooming, and cage exploratory behavior.
Social interaction	Rats engaging in allo-grooming and aggression behaviors, either on the giving or receiving side

The definitions are adopted from Hurst et al.[8]

2.4.2. Fecal sample collection

Fecal samples were collected from the animals once a week for six weeks at the same time in the morning using a method described by Parker et al.[25]. Each rat was gently removed from its cage and shortly handled for 2-3 minutes to facilitate the release of a fecal bolus. When this brief handling was ineffective, the rats were subsequently placed individually in a clean, empty cage, and freshly voided fecal pellets were carefully collected using sterile forceps, immediately transferred to labeled sterile tubes, and stored at -20 °C until further analysis[25].

2.4.3. Fecal corticosterone metabolite extraction

Fecal corticosterone metabolite extraction was performed in the Endocrine Research Laboratory at the Mammal Research Institute, University of Pretoria, South Africa, according to a method developed by Touma et al.[26]. The frozen fecal samples were freeze-dried for two days at -50°C and 0.96 mbar using a lyophilizer (CHRIST Alpha 1-2LD plus, TecsaReco, South Africa), and then ground with a pestle and mortar, and sifted through a mesh strainer to separate faecal powder and debris. A 0.10 g aliquot from each sample was transferred into a test tube containing 3 mL of 80% (v/v) ethanol. The mixture in each test tube was centrifuged at 2500 rpm for 10 minutes after being vortexed for 15 minutes. An aliquot of the supernatant (1.5 mL) was diluted (1:10) with assay buffer and transferred into a microcentrifuge tube, and frozen at -21°C until the time for corticosterone metabolite determination.

2.4.4. Corticosterone metabolite determination

Fecal corticosterone metabolite concentrations were measured using the 5a-pregnane-3b,11b,21-triol-20-one competitive enzyme immunoassay (EIA) method as described by Touma et al.[26]. The assay was performed using an Invitrogen progesterone competitive ELISA kit (Thermo Fisher Scientific, Massachusetts, USA). Anti-rabbit IgG-coated microtiter plates were used for the EIA. The antiserum was raised in rabbits against 5a-pregnane-3b,11b,21-triol-20-one (linked at position C20 to carboxymethyloxim) and coupled with bovine serum albumin. The same steroid linked at C20 to biotinyl-3,6,9-trioxaundecanediamin (EZ-Link Biotin-LC-PEO-Amine, Pierce, Rockford, IL, USA), served as the assay label. Established concentrations of the steroid standard (0.8-200 pg/well) were used to generate a standard curve. A mixture of the steroid standards (50 µl) or samples (50 µl) with the assay label (100 µl) and antibody (100 µl) was incubated in duplicate overnight at 4°C. Following incubation, the plates were washed four times with 0.02% Tween 20 washing solution (Art. No. 822184, Merck, Darmstadt, Germany) and blotted dry before 250 µl streptavidin horseradish peroxidase conjugate (¼ 4.2 mU, Art. No. 1089153, Boehringer, Mannheim, Germany) was added into each well. Plates were then left at 4°C in the dark on stirring tables for 45 minutes. After another washing step, 250 µl tetramethylbenzidine (¼ 69.4 nmol/well; Art. No. 87748, Fluka Chemika, Vienna, Austria) was added, and the plates were incubated for an additional 45 minutes at 4°C before the enzymatic reaction was stopped by the addition of 50 µl/well of 2 mol/L sulphuric acid. Absorbance was measured at a wavelength of 450 nm (reference filter: 620 nm) with an automatic plate reader (DigiScan, Asys Hitech GmbH, Eugendorf, Austria). The plate reader and Gen5 software measured the OD and provided an estimated hormone concentration for each sample. The final hormone concentrations were determined by the following formula[26].

FCM concentration (µg/g DW) = 20 × $V_{ethanol}$ (mL) × DF × C_{est} / Faecal weight (g) × 10^6.

DW is the dry fecal weight, V is the amount of ethanol (mL), DF is the dilution factor, and C_{est} is the estimated hormone concentration.

2.4.5. Animal weights

Rats were weighed weekly using a calibrated balance (Jadever scale model JWE-3K S/N W34911T0026, Labotec South Africa). At the end of the six-week period, the animals were euthanized using an overdose of isoflurane (Safeline Pharmaceuticals, South Africa)[27]. The carcasses were dissected, and the brain, thymus gland, spleen, and adrenal gland were collected and weighed using Soehnle Professional Scale 9437 S/N Fisher Scientific, Canada.

2.5. Statistical analysis

SPSS (version 27) was used for all statistical analyses. All the data set was checked for goodness of fit using the Shapiro-Wilk test. The mean behavior counts scored for intra- and inter-observer reliability testing were compared using the Kruskal-Wallis test. The Mann-Whitney U test was used to compare differences in the mean behavior counts of rats in the two cage types. The significance of differences in the means of FCM concentrations and organ weights between rats from the two housing systems was determined using an independent t-test. The weights of selected organs were expressed as relative weights of body weight. Although differences in mean FCM concentrations over time within groups were not expected, a repeated-measures ANOVA was used to assess their significance. Results were expressed as mean ± standard deviation (SD), and the level of significance for all statistical analyses was set at p-value equal to 5% ($p = 0.05$).

3. Results

3.1. Behavioral observations

Rats in semi-natural enriched cages were more active than in standard cages (Figure 2). The observer recorded significantly higher counts of enrichment-directed behaviors in semi-natural cages than in standard cages ($U = 617$, $p < 0.05$). Similarly, non-intake behaviors were significantly higher in semi-natural cages compared to standard cages ($U = 1908.5$, $p < 0.05$). In contrast, the counts of social interaction behaviors were significantly higher in standard cages than in semi-natural cages ($U = 2255$, $p < 0.05$). No difference was observed in intake behavior counts between the two cage types ($U = 2776$, $p > 0.05$; Table 2).

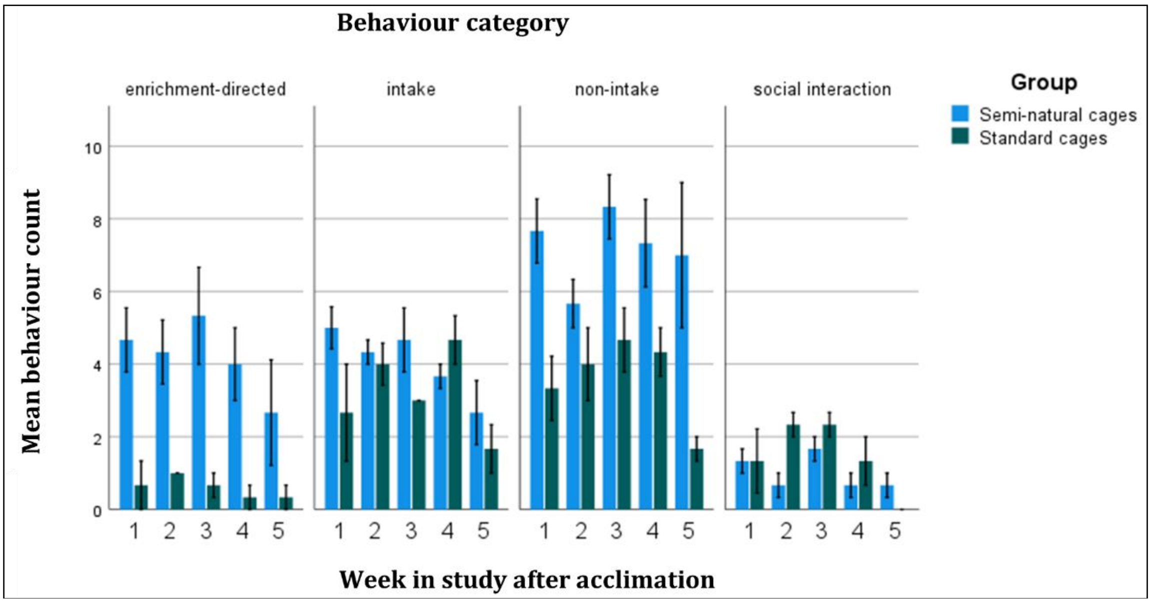

Figure 2. Mean behavior counts of 12-week-old rats housed in the standard and seminatural cages for five weeks

Table 2. Mean counts of behaviors observed in 12-week-old rats

Behaviour	Cage type	Mean counts	U statistic	P-value
Enrichment-directed	Semi-natural cage Standard cage	104.77^{a} 46.23^{b}	617.5	< 0.001
Intake	Semi-natural cage Standard cage	75.01^{a} 75.99^{a}	2,776	0.890
Non-intake activity	Semi-natural cage Standard cage	87.55^{a} 63.45^{b}	1,908.5	< 0.001
Social interaction	Semi-natural cage Standard cage	68.07^{b} 82.93^{a}	2,255.5	0.034

U statistic refers to the test statistic used in the Mann–Whitney U test. Values are expressed as mean ± SD. ^{a,b} Mean different superscript letters within the same behavior row indicated statistically significant differences between cage types at $p < 0.05$.

3.2. Growth and performance parameters

No significant differences in body weight were observed between rats housed in semi-natural cages and those kept in standard cages ($p > 0.05$). The weights of selected organs, including the brain, thymus gland, spleen, and adrenal glands, were reported as both absolute and relative weights (Table 3). Although the absolute spleen weight was higher in rats housed in standard cages (0.64 ± 0.04 g) compared to those in semi-natural cages (0.59 ± 0.02 g), the difference was not statistically significant ($p > 0.05$). Similarly, no significant differences were found in the absolute weights of the adrenal glands and brain between rats in standard cages (0.09 ± 0.005 g and 1.64 ± 0.05 g, respectively) and those in semi-natural cages (0.11 ± 0.006 g and 1.65 ± 0.03 g, respectively; $p > 0.05$). When the weights of the brain, spleen, and adrenal glands were expressed as percentages of carcass weight, no differences in relative organ weights were detected between the two housing systems.

Table 3. The absolute and relative weights of selected organs in 12-week-old rats

Organs	Standard cage	Semi-natural cage	P-value
Body weight (g)	289.4 ± 12.3	287.6 ± 11.8	0.721
Absolute weights of organs (g)			
Brain	1.64 ± 0.05	1.65 ± 0.03	0.584
Thymus gland	0.27 ± 0.02	0.27 ± 0.02	0.936
Spleen	0.64 ± 0.04	0.59 ± 0.02	0.087
Adrenal glands	0.09 ± 0.005	0.11 ± 0.006	0.063
Relative weights of organ (% of body weight)			
Brain	0.628 ± 0.021	0.626 ± 0.019	0.812
Thymus gland	0.101 ± 0.008	0.099 ± 0.007	0.523
Spleen	0.236 ± 0.015	0.238 ± 0.013	0.746
Adrenal glands	0.037 ± 0.002	0.039 ± 0.003	0.089

Values are expressed as mean ± SD. P-values derived from an independent t-test comparing standard versus semi-natural cages. No significant differences were observed for any parameter ($p > 0.05$ for all comparisons).

3.3. Fecal corticosterone metabolite concentrations

Mean FCM concentrations of rats housed in the two cage types are presented in Table 4. During the acclimation period (week 0), FCM levels were similar between rats in semi-natural cages (4.62 ± 2.08 µg/g DW) and standard cages (4.88 ± 0.88 μg/g DW; $p > 0.05$). In week 1, mean FCM concentrations were slightly higher in standard cages (6.59 ± 2.61 μg/g DW) compared to semi-natural cages (5.58 ± 1.15 μg/g DW), but the difference was not statistically significant ($p > 0.05$). Weeks 2 to 4 showed fluctuating FCM levels in both groups, with no significant differences observed between the housing types ($p > 0.05$). In week 5, rats housed in semi-natural cages exhibited significantly higher mean FCM concentrations (6.59 ± 1.65 μg/g DW) than those in standard cages (5.02 ± 1.17 μg/g DW; $p < 0.05$). By week 6, mean FCM levels were similar between semi-natural (6.09 ± 1.73 μg/g DW) and standard cages (6.08 ± 1.05 μg/g DW; $p > 0.05$). Average FCM concentrations indicated periodic fluctuations and an overall upward trend over time in both housing systems. Mauchly's test indicated that the assumption of sphericity was violated ($p < 0.05$). Despite the observed temporal variation, the effect of time was not statistically significant ($F = 1.59$, df = 4.27, 68.24, $p > 0.05$).

Table 3. Fecal corticosterone concentrations of 12-week-old rats housed in semi-natural cages and in standard cages

Week in study	Group (cage type)	Mean FCM concentration (μg/g DW)	t statistic	Degrees of freedom	P-value
Week 0 (at the end of acclimation)	Semi-natural cage Standard cage	4.62 ± 2.08 4.88 ± 0.88	-0.344	16	0.736
Week 1	Semi-natural cage Standard cage	5.58 ± 1.15 6.59 ± 2.61	-1.06	16	0.303
Week 2	Semi-natural cage Standard cage	5.75 ± 1.44 5.41 ± 1.69	0.46	16	0.648
Week 3	Semi-natural cage Standard cage	5.70 ± 1.14 5.48 ± 1.33	0.392	16	0.700
Week 4	Semi-natural cage Standard cage	6.56 ± 2.62 5.54 ± 0.62	1.13	16	0.272
Week 5	Semi-natural cage Standard cage	6.59 ± 1.65^{a} 5.02 ± 1.17^{b}	2.32	16	0.034
Week 6	Semi-natural cage Standard cage	6.09 ± 1.73 6.08 ± 1.05	0.020	16	0.984

FCM: Fecal corticosterone metabolite concentration, DW: Dry weight. t statistic: Determine whether there was a statistically significant difference between the means of FCM concentration in the standard cages and the Semi-natural cages, df: degrees of freedom. Values expressed as mean ± SD. ^{a,b} Different superscript letters within Week 5 indicate a statistically significant difference between cage types ($p < 0.05$). No other weekly comparisons showed significant differences.

4. Discussion

The use of the low-cost semi-natural caging system in the present study was motivated by current evidence that standard cages did not adequately meet rodents' behavioral and physiological needs[1]. It was believed that providing rats with an enriched environment, similar to the complexity of a pet rat cage, would improve animal welfare. Similar to the current experiment, the incorporation of complex pet-style rat cages into laboratory settings has been proposed by previous studies[12,28]. Although environmental enrichment strategies are usually used to encourage natural behaviors and behavioral diversity to improve animal welfare, the present study demonstrated that enrichment can sometimes have negative effects. These findings were consistent with those of Domínguez-Oliva et al.[29], who documented unintended negative outcomes associated with environmental enrichment. Some of the reported unintended negative outcomes included physical harm to animals, such as entanglement and trauma[30], exposure to pathogens, especially via organic enrichment material such as burrowing substrate, increased aggression due to competition over enrichment resources[29], and anxiety resulting from excessive stimulation[30]. Other studies have suggested that the effects of environmental enrichment can vary with the strain, age, and sex of the animals[32,33]. The present results indicated that semi-natural enriched cages stimulated locomotor activity and behavioral diversity and had no adverse effects on growth parameters or the HPA stress response. The increased frequency of enrichment-interaction and non-intake behaviors in semi-natural housing suggested that the rats in these environments experienced improved activity levels and greater behavioral diversity than standard laboratory cages. This finding was consistent with those of Bailoo et al.[33], Ratuski and Weary[34], Hendershott et al.[35], and Simpson and Kelly[36], who reported that environmental enrichment stimulated engagement with enrichment items and behavioral diversity. Collectively, these studies in rats and mice consistently demonstrated that enriched rodents exhibited higher levels of enrichment-directed behavior, such as exploration, object manipulation, nest building, and climbing, and these behaviors were quantitatively greater than in standard housing. In the present study, the semi-natural cage environment provided the rats with foraging opportunities and space to engage in natural locomotor activities, such as running and climbing. Previous studies have demonstrated that increased locomotor activity can enhance cellular stress resistance through the activation of NRF2-mediated antioxidant defense pathways[37]. In addition, environmental enrichment has been demonstrated to boost hippocampal-dependent learning and memory, restore synaptic plasticity, and consequently help preserve cerebral functions[38]. Consistent with these findings, the present study observed greater locomotor activity and behavioral diversity in rats housed in semi-natural cages, implying that such conditions may improve their ability to cope with captive stressors and challenges. Such physiological fluctuations might stem from factors specific to the individual animal, including diurnal secretion patterns, the stage of the estrous cycle, and metabolic processes[26].

Limited enrichment structures in standard cages may explain the higher counts of social interaction behavior observed in those cages. Food hoppers and waterspouts were among the only physical structures available to the rats in standard cages, and rats were frequently observed at these sites, either lying under them, interacting with them, or feeding from them. In standard cages, rats frequently visited the food hoppers and waterspouts, which may have increased opportunities to meet and interact, leading to higher social interaction counts. These results were consistent with the findings of Abou-Ismail[39], who reported that laboratory rats were thigmotactic and spent most of their inactive periods in contact with physical structures, such as surrounding walls or enrichment items in their environment. Because rats are prey animals, providing environmental enrichments that promote thigmotaxis reduces the time they have to spend looking out for predators and improves their ability to exert control over their environment[36]. Similar thigmotactic behavior was observed in semi-natural cages during the present study, with rats lying inside or against the provided enrichment structures. Laboratory animal rooms are often maintained at 20-24°C, which is below the adult rat's thermoneutral zone of approximately 28-30°C[40]. Shelters and nesting materials may help to create a microenvironment that supports heat conservation, as standard laboratory temperatures are typically below the rat's thermoneutral zone, suggesting a thermoregulatory function. Patterson-Kane et al.[41] reported that laboratory rats retain natural burrowing and nesting behaviors similar to those of their wild counterparts, further supporting the interpretation that shelter use represents normal behavioral expression.

Although body weight and organ weights can be useful indicators of animal welfare, their interpretation presents certain challenges. These parameters are influenced by multiple interacting factors, including nutrition, strain, age, social dynamics, activity levels, and environmental temperature[42]. The differences in body weight across different housing systems may indicate variations in energy expenditure or behavioral activity, rather than compromised welfare[7]. Similarly, organ weight changes can reflect adaptive physiological responses rather than poor welfare. In the present study, environmental enrichment in the semi-natural cages did not affect the rats' body weights. The present results are inconsistent with those of Spangenberg et al.[15] and Konkle et al.[18], who found decreased body weights in rats housed in enriched cages with additional space and physical objects. Rats housed in cages with additional space and physical objects may be more physically active, which can reduce fat deposition or slow weight gain compared to those housed in standard cages[15]. The similar body weights across cage types suggest that semi-natural cages did not harm the rats' welfare. Any negative effects may have been too minor to be detected by weight changes. Additionally, rats in semi-natural cages may not have been active enough to lose weight. The present findings contrast with those of Tsai et al.[43] and Abou-Ismail[39], who reported elevated body weights in rats housed in enriched standard cages compared with those in barren standard cages. There were no differences in relative organ weights in the present study, consistent with the findings of Spangenberg et al.[15], and Konkle et al.[18], who investigated the effects of pen housing on physical activity and fitness in Sprague-Dawley rats. Spangenberg et al.[15] reported no differences in relative organ weights between enriched and standard cage-housed rats. When interpreted within the context of animal welfare, the findings on relative organ weights may suggest that rats housed in both cage systems did not experience poor welfare or chronic stress. Prolonged stress is commonly associated with adrenal hypertrophy and thymic involution; however, such alterations may also reflect adaptive physiological responses to environmental conditions rather than compromised welfare alone[16].

Differences in FCM concentrations between the two cage types were not considerable at any sampling point during the present study period, except in week five. This finding indicated that the stress levels or stress responses experienced by the rats under the present experimental conditions were insufficient to produce notable elevations in FCM concentrations. In other words, neither standard cages nor semi-natural cages, as implemented in the present study, sufficiently affected FCM concentrations. These findings are consistent with Eriksson et al.[44], who reported that single-housing in metabolic cages did not produce detectable FCM elevations, whereas surgery, a more severe stressor, did. Similarly, in the present study, neither standard nor semi-natural cages appeared to induce sufficient stress to cause consistent, detectable changes in FCM concentrations. The trend in FCM concentrations observed from week one to week six in the present study was consistent with trends reported previously by Dahlin st al.[45], who indicated that non-significant fluctuations in FCM levels may reflect natural variations in corticosterone excretion rather than stress-induced changes. Such physiological fluctuations might stem from factors specific to the individual animal, including diurnal secretion patterns, the stage of the estrous cycle, and metabolic processes[1] as well as from experimental variables, such as sampling time, sample quality, assay sensitivity, and cage microenvironment. The elevated FCM concentrations observed in semi-natural cages during week five remain unexplained, as it was not associated with any identifiable stressful intervention.

5. Conclusion

Semi-natural cages assessed in the present study provided rats with opportunities to engage in increased natural locomotor activity and to express behavioral diversity, characteristics that may improve animal welfare. The cage modification implemented in the present study was affordable, practical to use, and did not compromise the rats' physical health or hinder checking the animals. The current findings supported the use of semi-natural cage housing when room space and the study parameters exist. The present study would allow facility managers and veterinary staff to evaluate the acceptability of these substrates in semi-natural cages in relation to the interval between cage changes, the health of laboratory animals, and their ability to meet the investigators needs. The present study had several limitations, including the long interval between fecal collections, which prevented detection of short-duration corticosterone peaks, unmonitored estrous cycles in the female rats, and the use of a single age group. Future studies should include both sexes, extend the study duration, monitor oestrous cycles, increase faecal sampling frequency, directly measure cellular stress and cerebral function, test different age groups, and systematically assess potential negative outcomes of enrichment such as aggression or pathogen exposure.

Declarations

Competing interests

The authors declare that they have no competing interest.

Availability of data and materials

The data of the manuscript is available on request from the corresponding author.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been thoroughly reviewed. No AI-assisted technologies were used in the generation of this manuscript.

Authors' contributions

Richard Mavunganidze performed the experimental analysis, interpreted the results, and drafted the manuscript. Vinny Naidoo supervised the study and edited the final edition of the manuscript. All authors have read and approved the final edition of the manuscript.

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