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Effects of Individual Cage Floor Area and Single Housing on Growth, Behaviour, Stress Responses and Health of Male Albino Rats

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ABSTRACT

The laboratory rats were widely used for the scientific research without much attention for their welfare especially the cage floor area available for them during their childhood period. Therefore, the current study was conducted to evaluate the effects of cage floor area on growth, behaviour, stress responses and health of Wister albino male rats from weaning to maturity. A total of 60 rats were used from 3 to 10 weeks old. The rats were divided into 4 groups; control group (630 cm^2/ rat), low cage floor area (157.5 cm^2 / rat), moderate cage floor area (315 cm^2 / rat), and single housing group (1260 cm^2 / rat), and left for one week for adaptation. Thereafter, the body weight (BW), body weight gain (BWG), feed intake, and water consumption were measured weekly. The behaviour of rats including total motor activity, locomotor and movement distance were measured by Actimeter. At the end of the experiment, all rats were slaughtered, and blood was collected for either blood smear performing or for determination of serum biochemicals and corticosterone (CORT).

The results showed that single housing of rats resulted in a reduced BW and BWG. Moreover, total motor activity, locomotor activity, and movement distance of rats were numerically decreased due to low cage floor area and single housing. Furthermore, crowding stress resulted in higher neutrophils to lymphocytes ratio (N / L ratio), higher level of serum CORT, and elevated serum high density lipoprotein (HDL) and alanine transaminase (ALT). These results suggest that crowding stress led to higher stress responses and adversely affect their health. In conclusion, crowding stress produced severe adverse effects for growth, behaviors, stress responses and health of rats. Besides, single hosing social stress under our experimental condition adversary altered the growth and behavioral measurements of rats without affecting their stress responses and health.

Keywords: *Welfare, Cage floor area, Social stress, Single housing, Rats.*

INTRODUCTION

Welfare assessments of animals have received much attention, especially farm animals and poultry. However, the welfare of laboratory animals used for scientific research is still to be evaluated. Laboratory rats are used as experimental animals for a wide branch of sciences. For example, they are used for basic scientific research, to develop and investigate a new medicine or treatment and to study the toxicological and safety range of substances. In addition, rats are used for drug discovery studies to reach a correct treatment for human and animal diseases. Mostly, rats are used without much focus on their welfare during the experimental period. Indeed, the welfare parameters of rats during the experiment are widely ignored by scientists. Nowadays, most universities have legislation for the use of animals in research experiments globally. This big development concerning laboratory animal welfare is coming from the efforts of animal welfare scientists to pay attention of the scientists to follow the welfare ethics of animals before performing research experiments using laboratory rats. Therefore, scientists of animal welfare are strongly focusing not only on the judgment of the use of laboratory animals but also on increasing the welfare status of laboratory rats. It is generally accepted that the harmony between animals and their living environment is the main concept of animal welfare (Koolhaas, 2011; Jirkof et al., 2019). Additionally, Neville et al., (2021) mentioned that growth, behavior, physiology and health are indicators for the assessment of welfare status of laboratory rats during their keeping for scientific purposes. The cage floor area per rat may adversely affect those welfare indicators due to the possibility of occurrence of crowding stress (Barker et al., 2017). However, little is known about cage floor area per individual rat during scientific experiments, especially during rearing period from weaning to adulthood. In addition, rats are social animals and prefer to live in groups; however, some researches are performed using individual housing of rats which contributes an isolation social stress. Both crowding and isolation stress may adversely influence the welfare of status of rats during experiments. Therefore, the current study was carried out to investigate the effects of cage floor area for each rat and single housing on growth, behaviors, stress responses, and health parameters of young male albino rats reared from weaning to maturity.

MATERIALS AND METHODS

Animals, housing, feeding and drinking

A total of sixty male Wistar albino rats of 3 weeks old were of average body weight of rats was 57 to 61 g and randomly housed in wire cages (45 × 28 × 16 cm, length, width and height respectively). Each cage was provided with a feeder, a glass bottle drinker with a rubber nibble, and 2 cm wood shaving as bedding. The temperature inside the laboratory was kept at 23

± 1 ̊c. The average relative humidity was kept at 40 to 50 %. The lighting was 12 hours, and 12 hours of darkness. The rats were randomly divided into 4 experimental groups; control group, 2 rats in each cage (630 cm^2 floor area for each rat) with six replicates (n = 12), low cage floor area group (8 rats in each cage, 157.5 cm^2 floor area for each rat) with three replicates (n = 24), moderate cage floor area group (4 rats in each cage, 315 cm^2 floor area for each rat)) with four replicates (n = 16), single housing (one rat in each cage, 1260 cm^2 floor area for each rat), with eight replicates (n = 8). All rats were housed for adaptation period without disturbance for one week. Feed and water were available *adlibitum*. A standard rat diet according to Reeves *et al.*, (1993) (5% fiber, 4% corn oil, 14% casein, 10% sucrose, 3.5% mineral mixture, 1% vitamin mixture, 0.25% choline chloride, 0.3% D-L methionine, and 61.95% corn starch) was offered for rats.

Measurements of growth, feeding and drinking

The body weight (BW) of each rat was taken weekly from four weeks old. The amount of feed and water were calculated for each experimental group starting from four weeks old after the adaptation period of one week. Therefore, feeding intake, water consumption and body weight gain (BWG) were measured weekly from 5 weeks old till 10 weeks old.

Behavioral measurements by Actimeter

Using Actimeter device (pan lab infrared Actimeter, Figure 1), the number of total motor activities (n / 10 min), number of locomotor activities (n / 10 min), and the movement distance (cm / 10 min) were measured for rats of each group.

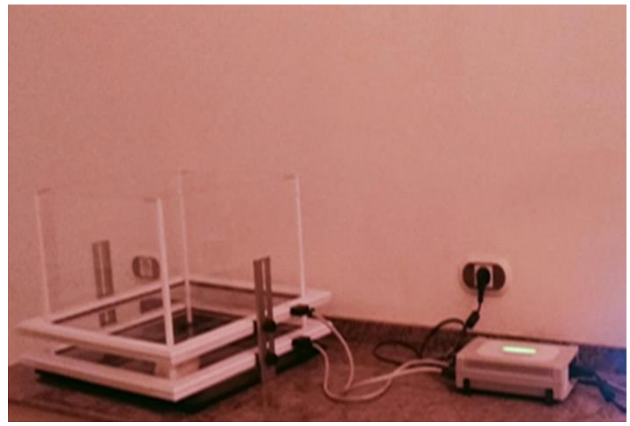

Fig. 1: photo of the pan lab infrared Actimeter device

Slaughtering and samples collection

At the end of experiment, all rats were weighed and slaughtered after inhalation with diethyl ether 99.9 %. The blood samples from each rat were taken from the inter-orbital plexus and the heart and collected into clean, dry, labeled tubes without anti-coagulant and centrifuged at 3000 rpm for 15 minutes for serum collection. The serum samples were stored at - 80 °C using Eppendorf tubes till the time for chemical analysis. Additionally, a blood drop of each rat was smeared on glass slide for differential white blood cell counting using light microscope.

Clinical serum biochemical analysis

Serum glucose, triglyceride, cholesterol, HDL, total protein, albumin, globulin, urea, creatinine, alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP) were determined by ultraviolet spectrophotometer.

Measurement of differential leucocytic count

Blood drop from plexus vein of each rat was taken and smeared at clean glass slide then the slide left to dry, fixed by methyl alcohol then stained by Giemsa stain and was examined by light microscope for counting of neutrophils (N) %, lymphocytes (L) %, monocytes % (M), eosinophils % (E), basophils % (B). The neutrophils % was divided by lymphocytes % to obtain N / L ratio.

Determination of serum CORT (ng/ml)

Serum CORT was estimated using an Enzyme-linked immunosorbent assay (ELISA) test kit (Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103, USA).

Statistical analysis

The SPSS (Statistical Package for social sciences) version 25 was used for data analysis. To identify the significant differences between the experimental groups, a one-way analysis of variance (ANOVA) was conducted, followed by a least significant difference test (LSD). When the probability (P) value is ≤ 0.05, it was considered significant.

Ethics statement

The animal experiment was approved by the ethical research committee of the faculty of veterinary medicine, South Valley University (6a / 14.11.2020). All husbandry practices and euthanasia were performed with full consideration of animal welfare.

RESULTS

Effects of cage floor area and single housing on growth of rats

The BW of rats reared with single housing (one rat / 1260 cm^2) (66.25 ± 8.19, 87.56 ± 10.45, 121.44 ± 16.34, 163.7 ± 17.18, 201.86 ± 16.68 and 237.41 ± 17.51 g, respectively, Table 1) decreased significantly ($P < 0.05$) at 4, 5, 6, 7, 8 and 9 weeks old compared with control group (630 cm^2 per each rat) (101.21±2.54, 143.68 ± 3.26, 187.32 ± 4.74, 227.48 ± 5.57, 253.87 ± 5.99 and 283.98 ± 7.11 g respectively, Table 1). However, at the end of the experiment (10 weeks old), the BW of rats reared under single housing didn't differ significantly ($P = 0.121$) (269.18 ± 18.31 g, Table 1) from control group (300.61 ± 7.31 g). In contrast, the BW of low cage floor area group (one rat / 157.5 cm^2) (102.74 ± 2.31, 138.09 ± 2.69, 183.32 ± 3.59, 214.33 ± 5.29, 242.75 ± 5.85, 275.9 ± 6.39 and 293.49 ± 7.62 g, respectively, Table 1) didn't differ significantly ($P > 0.05$) at 4, 5, 6, 7, 8, 9 and 10 weeks old compared with control group (101.21 ± 2.54, 143.68 ± 3.26, 187.32 ± 4.74, 227.48 ± 5.49, 253.87 ± 5.99, 283.98 ± 7.11 and 300.61 ± 7.31 g respectively, Table 1). Similarly, the BW of moderate cage floor area group (315 cm^2 per each rat) (108.84 ± 1.88, 148.05 ± 2.71, 193.54 ± 3.85, 232.55 ± 5.02, 260.02 ± 5.57, 285.22 ± 7.29 and 301.04 ± 7.98 g respectively, Table 1) didn't differ significantly ($P > 0.05$) at 4, 5, 6, 7, 8, 9 and 10 weeks old compared with control group (101.21 ± 2.54, 143.68 ± 3.26, 187.32 ± 4.74, 227.48 ± 5.57, 253.87 ± 5.99, 283.98 ± 7.11 and 300.61 ± 7.31 g respectively, Table 1).

Table 1: Effects of cage floor area and single housing on BW (g) of male albino rats

Age (Week)	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing (one rat/cage) (n= 8)	P Value
4	101.19^{a} ± 2.54	102.74^{a} ± 2.31	108.84^{a} ±1.88	66.25^{b} ± 8.19	0.001
5	143.68^{ab} ± 3.26	138.09^{b}± 2.69	148.05^{a} ± 2.71	87.56^{c} ± 10.45	0.001
6	187.32^{a} ± 4.74	183.32^{a} ± 3.59	193.54^{a} ± 3.85	121.44^{b} ± 16.34	0.001
7	227.48^{ab} ± 5.57	214.33^{b} ± 5.30	232.55^{a} ± 5.02	163.69^{c} ± 17.18	0.001
8	253.87^{a} ± 5.99	242.75^{a} ±5.85	260.02^{a} ± 5.57	201.86^{b} ± 16.68	0.001
9	283.98^{a} ± 7.11	275.9^{a} ± 6.39	285.22^{a} ± 7.29	237.41^{b} ± 17.51	0.008
10	300.61 ± 7.31	293.49 ± 7.62	301.04 ± 7.98	269.18 ± 18.31	0.201

$^{a, b, c}$ Means of the same row with different superscripts are significantly different (P ≤ 0.05, ANOVA followed by LSD).

Furthermore, Table 2 shows that BWG of rats reared with single housing at 5 weeks old (21.31 ± 5.83 g) was decreased significantly (P = 0.001) compared with control group (42.47 ± 1.55 g). However, the BWG of rats reared with low and moderate cage floor area (42.98 ± 2.16, 47.88 ± 1.72, 35.35 ± 1.95 and 39.22 ± 1.36 g, respectively) did not (P > 0.05) differ significantly at 5 weeks old from control animals (43.92 ± 1.89 and 42.47 ± 1.55 g, respectively, Table 2). ANOVA also revealed that there no significant differences recorded between experimental groups at 6,7,8, and 9 weeks old (Table 2). Moreover, at the end of the trial (10 weeks old), the BWG of the rats reared with single housing (31.77 ± 3.81 g) was significantly increased (P = 0.042) compared with control, low and moderate cage floor area groups (16.64 ± 1.64, 17.59 ± 3.63 and 15.82 ± 2.59 g respectively, Table 2). These results suggested that BWG of single-housing rats was decreased by isolation stress at the beginning of the experiment (5 weeks old). However, adaptation had occurred during the following ages resulting in an increase in the body weight gain of rats reared with single housing compared with other groups.

Table 2: Effects of cage floor area and single housing on BWG (g) of male albino rats

Age (Week)	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing (one rat/cage) (n= 8)	P Value
5	42.47[a] ±1.55	35.35[a] ± 1.95	39.22[a] ± 1.36	21.31[b] ± 5.83	0.001
6	43.65 ± 4.89	45.23 ± 2.32	45.49 ± 1.68	33.89 ± 7.72	0.197
7	40.16 ± 5.50	31.01± 3.35	39.02 ± 1.57	42.25 ± 4.65	0.132
8	26.39 ± 1.61	28.42 ± 3.75	27.47 ± 2.29	38.16 ± 5.53	0.269
9	30.10 ± 1.79	33.15 ± 3.05	25.2 ± 3.19	35.55 ± 4.15	0.176
10	16.64[b] ±1.64	17.59[b] ± 3.63	15.82[b] ± 2.59	31.77[a] ± 3.81	0.042

[a, b] Means of the same row with different superscripts are significantly different (P ≤ 0.05, ANOVA followed by LSD).

Table 3 shows that the feed intake of rats reared with single housing at 5 weeks old, (69.27 ± 8.69 g) was significantly decreased (P = 0.001) compared with control, low and moderate cage floor area groups (108.75 ± 3.29, 104.89±1.09 and 110.51 ± 1.31 g, respectively, Table 3). However, feed intake of rats reared with low and moderate cage floor area groups (104.89 ± 1.09 and 110.51 ± 1.31 g, respectively, P > 0.05) didn't show significant differences when compared with control rats (108.75 ± 3.29 g, Table 2). At 6 weeks old, a similar effect was obtained. Indeed, feed intake of rats reared with single housing (92.82 ± 12.74 g, P = 0.012) showed a significant decrease compared with control, low and moderate cage floor area groups (133.18 ± 4.52, 164.13 ± 31.74 and 135.71 ± 1.86 g, respectively, Table 3). However, feed intake of rats reared with low and moderate cage floor area groups (164.13 ± 31.7 and135.71 ± 1.86 g, respectively) didn't show significant (P > 0.05) differences when compared with control rats (133.18 ± 4.52 g, Table 3). Interestingly, at age of 7 weeks old, the feed intake of rats reared with single housing (112.65 ± 11.72 g) tends to be decreased (P = 0.070) compared with control, low and moderate cage floor area groups (142.55 ± 3.62, 133.88 ± 4.07 and 143.87 ± 3.79 g, respectively, Table 3), however, no significant differences for feed intake were detected (P > 0.05) between control, low and moderate cage floor area groups (142.55 ± 3.62, 133.88 ± 4.07 and 143.87 ± 3.79 g, respectively, Table 3). Moreover, ANOVA revealed that no significant differences were recorded between experimental groups at 8, 9, 10 weeks old for BWG (Table 3). These results suggested that isolation stress decreased feed intake (feeding behavior) at the beginning of the experiment (at 5, 6 and 7 weeks old) resulting in a decrease of body weight gain despite their high cage floor area.

Similarly, water consumption of rats reared with single housing at age of 5 weeks old (57.25 ± 10.42 ml) was decreased significantly (P = 0.001) compared with control (137.17 ± 4.54 ml, Table 4). However, water consumption of rats reared with low and moderate cage floor area groups (141.75 ± 4.33 and 133.56 ± 2.31 ml, respectively) didn't differ significantly at age of 5 weeks old compared with control rats (137.17 ± 4.54 ml, P > 0.05, Table 4). Similarly, at 6 weeks old, water consumption of rats reared with single housing (116.57 ± 20.46 ml) was decreased numerically (P= 0.083) compared with controls (167.17 ± 4.83 ml). These data suggests that isolation stress (one rat per cage) affects the drinking behavior of rats during the first two weeks of the experiment. Indeed, the amount of consumed water was lowered due to individual housing of rats despite their housing with large cage floor area.

Table 3: Effects of cage floor area and single housing on feed intake (g) of male albino rats

Age (Week)	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing (one rat/cage) (n= 8)	P Value
5	108.75^{a} ±3.29	104.89^{a} ± 1.09	110.51^{a} ±1.31	69.27^{b} ±8.69	0.001
6	133.18^{a} ±4.52	164.13^{a} ±31.74	135.71^{a}±1.86	92.82^{b} ±12.74	0.012
7	142.55 ±3.62	133.88± 4.07	143.87 ±3.79	112.65 ±11.72	0.070
8	152.51 ± 6.22	146.06 ± 1.56	146.88 ± 2.56	143.78 ± 8.84	0.847
9	153.98 ± 6.37	151.86 ± 2.25	147.3 ± 1.01	136.31 ± 14.33	0.654
10	142.3 ± 6.56	154.66 ± 4.05	138.44 ± 2.59	149.55 ± 11.32	0.743

$^{a, b}$ Means within the same row with different superscripts are significantly different (P ≤ 0.05, ANOVA followed by LSD).

Additionally, low and moderate cage floor area (175.46 ± 3.58 and 154.44 ± 14.35 ml, respectively, Table 4) didn't significantly influence the amount of consumed water (P > 0.05) compared with controls (167.17 ± 4.83 ml, Table 4). Moreover, at 7, 8, 9 and 10 weeks old, water consumption of rats reared with low, moderate cage floor area and single housing groups (197.42 ± 2.79, 171.75 ± 24.98 and 157 ± 16.26 ml, 204.75 ± 5.12, 172.75 ± 26.37, 170.38 ± 12.13, 203.33 ± 6, 167.69 ± 22.01, and 176.38 ± 15.33, 205.38 ± 8.1, 161.81 ± 18.38, 184.88 ±11.01 ml respectively, P > 0.05, Table 4) didn't differ significantly compared with control rats (183.83 ± 4.36 ml, 183.92 ± 2.8 ml, and 184.99 ± 2.79 ml).

Table 4: Effects of cage floor area and single housing on water consumption (ml) of male albino rats

[a, b] Means within the same row with different superscripts are significantly different (P ≤ 0.05, ANOVA followed by LSD).

Age (Week)	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing (one rat/cage) (n= 8)	P Valie
5	137.17^{a} ±4.54	141.75^{a} ± 4.33	133.56^{a} ± 2.31	57.25^{b} ± 10.42	0.001
6	167.17 ± 4.83	175.46 ± 3.58	154.44 ±14.35	116.75 ± 20.46	0.083
7	183.83 ± 4.36	197.42 ± 2.79	171.75 ± 24.98	157 ± 16.26	0.367
8	183.92 ± 2.79	204.75 ± 5.12	172.75 ± 26.37	170.38 ± 12.13	0.434
9	184.99 ± 2.79	203.33 ± 5.43	167.69 ± 22.01	176.38 ± 15.33	0.573
10	171.83 ± 4.19	205.38 ± 8.1	161.81 ± 18.38	184.88 ± 11.01	0.176

Effects of cage floor area and single housing on behavioral measurements of rats

The number of total motor activity for rats reared under low, moderate cage floor area and single housing groups (1266.38 ± 121.66, 1419.25 ± 115.27 and 1485 ± 140.71 respectively, Table 5) was declined numerically compared with control rats (1629.38 ± 194.35). A similar effect was obtained for the number of locomotor activity. Indeed, rats reared under low, moderate cage floor area and single housing groups (1183.38 ± 112.08, 1317.13 ±108.49 and 1376.67 ± 129.14 respectively, P = 0.397, Table 5) showed a numerical decline compared with control rats.

Regarding the movement distance, a numerical reduction for rats reared under low, moderate cage floor area and single housing groups (1856.69 ± 209.99, 2140.31 ± 211.93 and 2286.77 ± 269.61 cm respectively) compared control rats (2574.68 ± 377.58 cm) (Table 5).

Effects of cage floor area and single housing on clinical serum biochemicals of rats

Interestingly, serum glucose level of rats reared under single housing (122.43 ± 8.64 mg/dl) was decreased numerically compared with control rats (145.14 ± 12 mg/dl) but the difference did not reach significance (P = 0.202, Table 6). Similarly, serum triglyceride level of rats reared under single housing (202.47 ± 31.65 mg/dl, P = 0.517) was numerically decreased compared with control rats (229.26 ± 28.37 mg/dl, Table 6).

In addition, the serum cholesterol level of rats reared under low, moderate cage floor area, and single housing groups (73.05 ± 4.51, 64.44 ± 5.37 and 73.83 ± 6.70 mg/dl respectively, Table 6) didn't differ significantly from control rats

Table 5: Effect of cage floor area and single housing on behavioral measurements

Behavioral measurements	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing (one rat/cage) (n= 8)	P Value
Total motor activity (n)	1629.38 ± 194.35	1266.38 ± 121.66	1419.25 ± 115.27	1485 ± 140.71	0.368
Locomotor activity (n)	1510.25 ± 181.63	1183.38 ± 112.08	1317.13 ± 108.49	1379.67 ± 129.14	0.397
Movement distance (cm)	2574.68 ± 377.58	1856.69 ± 209.99	2140.31 ± 211.93	2286.77 ± 269.61	0.321

(68.06 ± 4.17 mg/dl). Surprisingly, serum HDL of rats reared under low cage floor area (519.39 ± 15.97 mg/dl) was increased significantly (P = 0.026, Table 6) compared with control rats (449.26 ± 25.98 mg/dl). However, HDL of rats reared under moderate cage floor area and single housing groups (467.06 ± 26.32 and 424.44 ± 25.88 mg/dl respectively) didn't differ significantly from control rats (449.26 ± 25.98 mg/dl, P > 0.05, Table 6).

Table 6 shows also that serum total protein, globulin, albumin, albumin to globulin ratio, urea, and creatine did not differ between experimental groups. Furthermore, serum ALT showed a significant variation between the rats reared either under control, low cage floor area and single housing groups (36.47± 4.79, 41.15 ± 3.74 and 39.58 ± 5.03 IU/dl, respectively, Table 6). Indeed, serum ALT of rats reared under moderate cage floor area group (25.96 ± 3.20 IU/dl) tended to be decreased (P > 0.098, Table 6) compared with control rats (36.47± 4.79 IU/dl, Table 6). Moreover, serum ALT was numerically increased for rats reared under low cage floor area group (41.15 ± 3.74 IU/dl) compared with control rats (36.47± 4.79 IU/dl, Table 6).

However, serum AST of rats reared under low, moderate cage floor area and single housing groups (171.86 ± 15.29, 142.78 ± 15.06, 134.01 ± 24.77 and 134.01 ± 24.77 IU/dl, respectively, P > 0.05, Table 6) didn't differ significantly compared with control rats (167.86 ± 16.49 IU/dl). Similarly, serum ALP of rats reared under low, moderate cage floor area and single housing groups (144.53 ± 10.55, 140.44 ± 13.45 and 177.94 ± 24.81 IU/dl respectively, P = 0.194, Table 6) didn't differ significantly from control rats (122.72 ± 17.52 IU/dl, Table 6).

Table 6: Effects of cage floor area and single housing on clinical serum biochemicals of male albino rats

Parameters	Control (2 Rats / Cage) (n= 12)	Low cage floor area (8 rats/cage) (n= 24)	Moderate cage floor area (4 rats/cage) (n= 16)	Single housing group (one rat/cage) (n= 8)	P Value
Glucose (mg/dl)	145.14 ± 12.00	130.43 ± 7.43	139.19 ± 11.51	122.43 ± 8.64	0.538
Triglyceride (mg/dl)	229.26 ± 28.37	220.93 ± 19.7	237.62 ± 18.02	202.47 ± 31.65	0.832
Cholesterol (mg /dl)	68.06 ± 4.17	73.05 ± 4.51	64.44 ± 5.37	73.83 ± 6.7	0.553
HDL (mg/dl)	449.26 ± 25.98^{b}	519.39 ± 15.97^{a}	467.06 ± 26.32^{ab}	424.44 ± 25.88^{b}	0.025
Total Protein (g / dl)	6.72 ± 0.44	6.28 ± 0.29	6.01 ± 0.40	6.40 ± 0.91	0.749
Albumin (g / dl)	2.93 ± 0.14	2.93 ± 0.11	3.09 ± 0.10	2.83 ± 0.20	0.631
Globulin (g / dl)	3.79 ± 0.382	3.36 ± 0.327	2.97 ± 0.343	3.57 ± 0.967	0.637
A / G Ratio	0.88 ± 0.109	1.33 ± 0.26	0.50 ± 0.63	1.85 ± 0.61	0.223
Urea (mg/dl)	58.73± 3.66	56.82 ± 3.4	50.88± 2.92	58.6 ± 3.00	0.414
Creatinine (mg/dl)	0.56 ± 0.07^{ab}	0.74 ± 0.04^{a}	0.61 ± 0.05^{b}	0.59 ± 0.06^{ab}	0.081
ALT (IU / dl)	36.47 ± 4.79^{ab}	41.15 ± 3.74^{a}	25.96 ± 3.20^{b}	39.58 ± 5.03^{ab}	0.044
AST (IU / dl)	167.86 ± 16.49	171.86 ± 15.29	142.78 ± 15.06	134.01 ± 24.77	0.388
ALP (IU / dl)	122.72 ± 17.52	144.5 ± 10.55	140.44 ± 13.45	177.94 ± 24.81	0.194

[a, b] Means within the same row with different superscripts are significantly different ($P \leq 0.05$, ANOVA followed by LSD)

Effects of cage floor area and single housing on differential leucocytes count and N to L ratio

Interestingly, neutrophils % of rats reared under low and moderate cage floor area groups (35.40 ± 0.68 % and 26.40 ± 1.36 %, Table 7) were significantly increased (P = 0.001) compared with control and single-housed rats (16.40 ± 1. 38 % and15.60 ± 1.16 %). As well as lymphocytes % of rats reared under low and moderate cage floor area groups (57.80 ± 0.66 % and 70.20 ± 0.97 %, Table 7) were significantly decreased (P = 0.001) compared with control rats (79.40 ± 0.71%), however, no significant differences between lymphocytes % of control and single housed rats (79.40 ± 0.71% and 80.60 ± 0.93%) were recorded.

The monocytes % and Eosinophils % of rats did not show significant differences between groups (Table 7). In addition, basophiles % of rats reared under low and moderate cage floor area groups (3.80 ± 0.75 and 1.60 ± 1.02 %, Table 5) were significantly increased (P = 0.001) compared with control and single-housed rats % (0.00 ± 9.39 % and 0.20 ±1.05 %).

Surprisingly, N to L ratio of rats reared under low and moderate cage floor area groups (0.61 ± 0.017 and 0.38 ± 0.025, Table 7) were significantly increased (P = 0.001, Table 7) compared with control and single-housed rats (0.21 ± 0.021 and 0.19 ± 0.015).

Table 7: Effects of cage floor area and single housing on differential leucocytic count and neutrophils to lymphocytes ratio

	Control (2 Rats/Cage) (n=12)	Low cage floor area (8 rats/cage) (n=24)	Moderate cage floor area (4 rats/cage) (n=16)	Single housing (one rat/cage) (n=8)	P value
N %	$16.40^{c} \pm 1.38$	$35.40^{a} \pm 0.68$	$26.40^{b} \pm 1.36$	$15.60^{c} \pm 1.16$	0.001
L %	$79.40^{a} \pm 0.71$	$57.80^{c} \pm 0.66$	$70.20^{b} \pm 0.97$	$80.60^{a} \pm 0.93$	0.001
M %	2.40 ± 0.86	$1.60^{b} \pm 0.86$	$2.60^{c} \pm 0.51$	$1.80^{b} \pm 0.37$	0.322
E %	2.20 ± 0.74	1.40 ± 0.75	0.80 ± 0.37	2.20 ± 0.66	0.204
B %	$0.00^{c} \pm 9.39$	$3.80^{a} \pm 0.75$	$1.60^{b} \pm 1.02$	$0.20^{c} \pm 1.05$	0.001
N /L Ratio	$0.21^{c} \pm 0.021$	$0.61^{a} \pm 0.017$	$0.38^{b} \pm 0.025$	$0.19^{c} \pm 0.015$	0.001

[a, b, c] Means within the same row with different superscripts are significantly different ($P \leq 0.05$, ANOVA followed by LSD)

Effects of cage floor area and single housing on serum CORT of rats

Interestingly, serum CORT showed a significant difference between control rats, low, moderate cage floor area and single housing groups (P = 0.004, Figure 2). Indeed, serum CORT of rats reared under low cage floor area (133.78 ± 5.22 ng/ml, Figure 2) was significantly increased (P = 0.004) compared with control rats (104.84 ± 5.49 ng/ml, Figure 2). However, serum CORT of rats reared under single housing (99.96 ± 4.94 ng/ml, Figure 2) did not differ significantly (P = 0.694) from control rats (104.84 ± 5.49 ng/ml, Figure 2). Furthermore, serum CORT of rats reared under moderate cage floor area (116.31 ± 10.2 ng/ml, Figure 2) tended to be reduced (P = 0.059) when compared with rats reared under low cage floor area (133.78 ± 5.22 ng/ml, Figure 2).

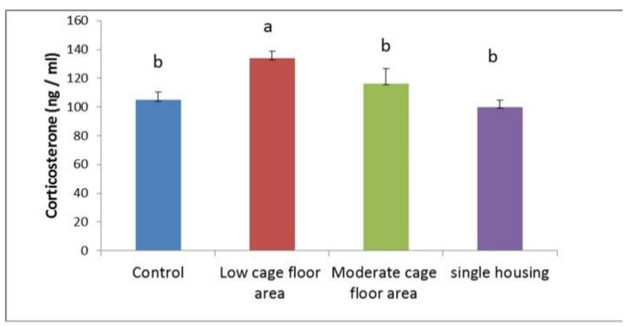

Fig. 2: Effects of cage floor area and single housing on serum corticosterone of male albino rats

DISCUSSION

The rat is a well-known animal model for the biomedical research experiments and the first mammal used for scientific research experiments (Wall and Shani, 2008). The welfare of rats during the scientific research is not only necessary from the animal welfare point of view but also from the accuracy and truthfulness of the obtained scientific results. Most importantly, the number of rats in a defined cage floor area requires to be evaluated carefully, given that overcrowding may produce adverse effects for rats and may impact the experimental outcomes (Bernatova et al., 2007). In addition, individual housing of rats may induce an isolation social stress resulting in an adverse impact on growth and behaviors of rats. Adult rats with cage floor area of 600 cm^2 per individual have been used for scientific experiments (Bean et al., 2008). However, individual cage floor area of young rats from weaning to adulthood and their single housing are to be evaluated. Therefore, the current experiment was conducted to investigate the effects of individual cage floor area and single housing on growth, behavior, stress responses and health parameters of male albino rats.

The results of the current research provide valuable information for validation of welfare indicators to assess the welfare of male rats reared with crowding stress and single housing.

In the current study, BW, BWG, feed intake, and water consumption do not alter by the cage floor area suggesting that cage floor area does not impact the growth from 4 to 10 weeks. On the other hand, growth may be not a valuable indicator to measure the stress of cage floor area alteration during this rearing period. This finding is in line with previous reports (Maslova, 2010; Schipper et al., 2018). These authors concluded that cage floor area may be not a potential stress factor that affects the growth.

However, single housing results in a reduction of BW and BWG, feed intake, and water consumption indicating that single housing is a potential stress factor. These findings agree with previous reports (Krohn et al., 2006). It is known that rat is highly social animal and live normally in a social group (Begni et al., 2020). Our results indicate that growth is a valuable indicator to measure the welfare of rats suffering from isolation social stress.

In this study, rearing of rats singly exposed them to isolation stress with higher cage floor area. Such isolation stress results in a reduction of their growth in terms of decreasing the BW, BWG, feed intake, and water consumption during the first weeks of experiments. However, at last week of experiment, their BWG was improved due to higher feed intake because of adaptation to their environment with time. The same results were reported previously (Nakhate et al., 2011). The model of isolation stress in this experiment was provided with larger cage floor area of 1260 cm^2 per rat which may reduce the adverse effects of isolation stress within time under the current experimental conditions. However, such results need to be verified with future research to find out the impacts of isolation stress with same cage floor area used for rats reared in group as a control (630 cm^2 per rat).

Moreover, the reduction of feed intake and water consumption due to isolation stress indicates that feeding and drinking behaviors were altered suggesting that ethological parameters can be used to measure welfare of rats during scientific experiments. It was shown that the behavioral indicator is a useful tool for measuring the welfare of animals (Leach and Main, 2008; Neville et al., 2021).

In an open field test using infrared Actimeter, the total motor activity, locomotor activity and movement distance of rats at 5 weeks old were numerically less either for rats reared with small cage floor area or those reared with isolation social stress. Similar results were published previously for either overcrowding social stress (Botelho et al., 2007) or isolation stress (Zorozo et al., 2019) using adult male rats. Such data may indicate that social stresses may interact with the exploratory behavior of rats. On other hand, it may indicate that those behavioral patterns might be partially used as an ethological indicator to measure the welfare of rats during scientific research at least during their childhood and adolescence period (from weaning to maturity age).

It was previously known that circulating CORT is a hormone to measure chronic stress in mammals (Kirillov et al., 2003; Bean et al., 2008; Helal et al., 2014). In the present study, circulating CORT concentration as a physiological response to chronic stress was measured at the end of experiment. The results showed that overcrowding or rearing rats with low cage floor area (157.5 cm^2 per rat) increased the level of circulating CORT compared with rats reared either with 630 cm^2 per rat (control) or 315 cm^2 per rat or 1260 cm^2 per rat suggesting that reducing the cage floor area is a powerful stress factor during rearing of rats for scientific experiment. It is suggesting that serum CORT level is a powerful stress response and a significant physiological indicator to measure the welfare of rat during their early stages of life. This result is highly significant for scientists using rats for their laboratory to avoid the potential occurrence of chronic social stress during performing their researches. Increasing the level of circulating CORT may not only affect the accuracy of scientific experiment but also may lead to immune-suppression and many other adverse impacts on health (Coutinho and Chapman, 2011). For example, stress increased the total white blood cell count (Davis et al., 2018) and increased the individual white blood cells (neutrophils, basophils, lymphocytes, eosinophils, and monocytes) (Yıldız et al., 2007) due to overcrowding of rats. A similar result was obtained in our experiment. Overcrowding stress resulted in a higher percentage of neutrophils and basophils and a lower percentage of lymphocytes in the current study.

Moreover, N to L ratio in present experiment was significantly increased for rats reared with low cage floor area (157.5 cm^2 per rat) compared with rats reared either with 630 cm^2 per rat or 315 cm^2 per rat or 1260 cm^2 per rat indicating that overcrowding stress during rearing period of rats adversely impacted the welfare of rats.

Interestingly, N to L ratio in this study was influenced by cage floor area available for each rat in a similar manner as circulating corticosterone suggesting that N to L ratio can be used as physiological stress response for rats. It was previously shown that N to L ratio was a critical stress indicator for rodents (Yıldız et al., 2007) and for mammals (Davis et al., 2018; Seltmann et al., 2020). Higher level of stress responses in the terms of elevated corticosterone and N to L ratio due to overcrowding social stress may adversely affect clinical serum parameters as well as liver and kidney enzymes resulting in an alteration of rat health parameters (Gamallo et al., 1986; Shawer et al., 2016).

In the present study, overcrowding social stress (157.5 cm^2 per rat) increased the level of serum ALT compared with controls (630 cm^2 per rat) or 315 cm^2 per rat or 1260 cm^2 per rat. Similarly, HDL was significantly elevated due to overcrowding social stress indicating that overcrowding as a chronic stress affected the protein and lipid metabolism. Those results are in line with previous reports (Willis et al., 2009; Shawer et al., 2016). Such results suggest that overcrowding stress adversely affect the liver function and health.

CONCLUSION

From the results of this study, it can be concluded that growth parameters such as BW, BWG, feed intake, and water consumption can be used for measuring the welfare of Wister male albino rats reared from weaning to maturity due to its efficacy to measure isolation stress. Moreover, the locomotion behavior of rats in an open field test can be used partly for estimating the welfare of Wister male albino rats and partially could measure social stresses (crowding and isolation). Furthermore, CORT and N / L ratio can be used to assess the welfare of Wister male albino rats. Additionally, serum HDL, creatinine and ALT in addition to the percentages of neutrophils, basophiles and lymphocytes may be used as health indicator to measure the welfare of Wister male albino rats.

To summarize, crowding stress has significant adverse impacts on stress responses including CORT hormone and N to L ratio. On the other hand, single housing of rats under the current experimental condition adversely influenced the growth and behavior of rats without significant impacts on stress responses, and health. This may be due to adaptation of rats to the model of isolation within time or due to the wide cage floor area (1260 cm2 per rat) available for each rat during isolation.

Overall, social stresses either crowding housing or single housing adversely affected the welfare of rats during their rearing period from weaning to maturity. Therefore, it is advisable for the researchers using male albino rats during age of weaning to maturity to pay attention for the concern of welfare assessment to avoid the impact of social stresses on the welfare of rats which may adversely affect the truth of their results.

CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

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