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# Physiological Profile of Growing Rats: Effects of Cage Type and Cage Density

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**ABSTRACT :** This experiment was conducted to examine the effects of cage type (CT) and cage density (CD) on physiological variables in growing rats. Sprague Dawley rats (n = 108) weighing an average of 46 g were housed in metallic cage with woodchip bedding (MCWB), metallic cage with wire mesh (MCWM), and plastic shoebox with woodchip bedding (PCWB) separately by sex at normal (160-cm$^2$/rat, ND) and high (80-cm$^2$/rat, HD) CD from 3 to 10 wks of age. All cages were in dimension of 24×40×20 cm (W×D×H). At the end of the experiment, blood samples were collected and 6 rats from each cage were sacrificed. No death was observed among rats at ND, whereas mortality rate at HD was 22.3% for males and 13.9% for females. Heart weight was affected by CT. Doubling CD caused 23, 11.8, 17.9, 8.6, 6.9, and 16.4% decreases in BW and weights of heart, liver, kidney, testis, and ovary, respectively. Except for adrenal gland, other organs for males were heavier than for females. Liver weight of males and females responded differently to CT and CD. Comparing with females, males had 7.3 and 5.2% heavier and 9.9% lighter liver weights in MCWB, MCWM, and PCWB, respectively. As CD doubled, liver weight for males and females decreased by 22.4 and 13.1%, respectively. Mean adrenal gland weight increased by 8.4% and decreased by 9.7% for males and females, respectively, with doubling CD. CT affected glucose, TG, Ca, and ALP levels. However, CD did not alter blood chemistry. Rats housed in metallic cages had greater neutrophil count and neutrophil:lymphocyte ratio than rats housed in plastic cages. Doubling CD caused a 24.2% increase in lymphocyte count. There were CT by CD, CT by sex, and CD by sex interaction effects on lymphocyte count. Doubling CD caused 0.1% decrease and 49.8 and 26.7% increases in lymphocyte count for rats housed in MCWB, MCWM, and PCWB, respectively. Comparing with females, lymphocyte count for males housed in MCWB, MCWM, and PCWB had 8.9 and 12.9% greater and 30.3% less lymphocyte counts, respectively. Lymphocyte count decreased by 4.12% for males, whereas it increased by 61.0% for females as CD doubled. Doubling CD resulted in 2.5 and 2.3% increases in erythrocyte count and hematocrit value. These data suggest that animals perform better in metallic cages than in plastic cages and that cage density had pronounceable effects on physiological parameters in a cage type and sex dependent-manner. (**Key Words :** Rat, Cage Type, Cage Density, Sex, Growth, Organ Development, Blood Metabolite, Hemogram)

## INTRODUCTION

Laboratory animals are frequently used in experimental studies to contribute scientific knowledge in various research areas. Results generated from laboratory animals are often extrapolated to address medical issues. However, intensive use of laboratory animals raises ethical issues. The welfare of an animal in response to husbandry practices can be assessed by evaluating efforts to be made by the animal in order to deal with conditions for maintaining normal growth and health status (Barnett and Hemsworth, 2003). Provision of a comfortable area to allow normal postural and behavioral adjustments is important for the animal and the outcome of the animal experiments (Monteiro et al., 1989; Woolverton et al., 1989; Baumans, 2005). As a result of failure to provide comfort, growth and organ development may be depressed and well-being of animals may be compromised, as reflected by hematological variables and hormone status (Klir et al., 1984; Monteiro et al., 1989).

Information on animal responses to cage type (CT) with

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**Table 1.** Ingredient and nutrient composition of the diet

| Item | % | Nutrient[1] | % |
|---|---|---|---|
| Corn | 38.50 | Dry matter | 89.47 |
| Rye | 10.70 | Crude protein | 23.65 |
| Wheat bran | 4.00 | Crude fiber | 3.41 |
| Soybean meal | 35.00 | Ether extract | 5.66 |
| Sunflower meal | 4.30 | Ash | 7.54 |
| Fish meal | 2.50 | Methionine | 0.53 |
| Sunflower oil | 2.80 | Lysine | 1.29 |
| Limestone | 1.00 | Threonine | 0.90 |
| Salt | 0.30 | Leucine | 1.88 |
| DL-methionine | 0.15 | Arginine | 1.58 |
| $NaHCO_3$ | 0.50 | Ca | 1.24 |
| Vitamin-mineral premix[2] | 0.25 | P | 0.99 |

[1] Calculated from tabular values.

[2] Per kg contains: vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU, vitamin E (dl-α-tocopheryl acetate), 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin $B_{12}$, 15 mcg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg.

respect to cage material and flooring is limited. It appears that metallic cages preserve body heat more efficiently than plastic cages (Gordon and Fogelson, 1994). Use of bedding material as compared with wire mesh floor provides better environment for physical activity such as dwelling and jumping (Manser et al., 1996; Weerd et al., 1996). The utilization of bedding materials (e.g., paper, woodchip, ground corncob), however, puts the animals in contact with their excreta and compromises airflow (Raynor et al., 1983). Crowding interferes with physical activity and psychological needs and also compromises feed intake and growth (Renne, 1989; Restrepo and Armario, 1989), and consequently suppresses immune system and well-being (Rock et al., 1997) of males (Arakawa, 2005) and females (Eskola and Kaliste-Korhonen, 1999). For rats heavier than 150 g, 150-$cm^2$ area per rat is recommended (CCAC, 1993). A large retrospective study involving chickens showed that the adverse effect of increasing cage density (CD) was not due to crowding per se, but due to other related housing conditions (e.g., immobilization, heat, humidity, and accumulation of gaseous compounds) (Dawkins et al., 2004). This conclusion may be valid for laboratory animals (Keller et al., 1989; Reeb-Whitaker et al., 2001). The objective of this experiment was to evaluate the effects of CT, CD, and sex on growth, organ development, blood parameters, and hemogram measurements from the post-weanling to puberty period in rats.

## MATERIALS AND METHODS

### Animal and experimental group

The Research Animal Ethic Committee of Atatürk University approved the protocol under this experiment. Fifty-four male and 54 female Sprague Dawley rats weighing an average of 46.4 g (42.2-51.2) were obtained from Atatürk University Experimental Animal Teaching and Research Center (ATADEM Breeding Facility). Animals were routinely subjected to microbiological evaluation for major pathogens (*Salmonella spp.*, *Shigellae spp.*, *Leptospira spp.*, *Streptobacillus moniliformis*, *Spirillum minus*, *Mycobacterium tuberculosis*, *Pastorella pseudotuberculosis*, and *Sarcoptes scapiei*). Rats were allocated to metallic (aluminum) cage with woodchip bedding (MCWB), metallic cage with wire mesh (MCWM), and plastic shoebox (polycarbonate) with woodchip bedding (PCWB) (24×40×20 cm, W×D×H for all cages) at normal (160-$cm^2$/rat or 6 rats/cage, ND) or high (80-$cm^2$/rat or 12 rats/cage, HD) CD according to sex from 3 to 10 wks of age. That is, 3×2×2 factorial allocation was arranged according to sex to contain 6 and 12 rats at ND and HD in each of three CT.

### Diet and management

After the weaning period (wk 3), rats were switched to *ad libitum* consumption of the conventional pellet diet (Table 1) formulated to meet nutrient requirements (NRC, 1995). During the experiment, temperature and humidity were maintained at 20-24°C and 58%. All rats were exposed to 12:12 light:dark cycle (CCAC, 1993). Cages were cleaned twice a week. Water was always available via glass bottles with rubber nipples.

### Sample collection and analytical procedure

At the end of the experiment, rats were fasted 24 hrs before weighing final body weight (BW) and sampling blood from heart under anesthesia. Blood samples were put into additive-free vacutainers (BD vacutainer SST, BD Vacutainer Systems Preanalytical Solutions, Belliver Industrial Estate, London, UK) for serum chemistry and vacutainers with $K_3$-EDTA for hemogram. Serum was obtained following centrifugation at 3,000 g for 15 min at 20°C. Aliquots were kept at -20°C until analyses for glucose, total protein (TP), albumin, creatine, triglyceride (TG), cholesterol, very low-density lipoprotein (VLDL), alkaline phosphatase (ALP), Ca, and P using spectrophotometric methods with commercial kits (DDS, Diasis Diagnostic Systems Co., İstanbul, Turkey). Within one hour after the collection, whole blood samples were subjected to flow-cytochemistry (Coulter STKS™ Hematology Flow Cytometer, Beckman Coulter Co., Miami, FL, USA) for neutrophil, lymphocyte, eosinphile, basophile, erythrocyte, and platelet counts, hemoglobin concentration, and hematocrit value.

After sedation by intramuscularly injection of xylazine hydrochloride (5 mg/kg) (Rompun, Bayer, İstanbul, Turkey), rats were placed into anesthesia box sprayed with 2% sevoflurane (Sevorane, Abbott Laboratories, İstanbul,

**Table 2.** The effects of cage type, cage density, and sex on body weight and organ weights in growing rats

| Groups[1] | | | Body weight and organ weights[2] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CT | CD | S | BW (g) | Heart (g) | Lung (g) | Stom (g) | SI (cm) | LI (cm) | Liver (g) | Kidney (g) | Spleen (g) | AG (g) | Testis (g) | Ovary (g) |
| MCWB (n=36) | | | 177.9 | 0.77[a] | 1.02 | 1.16 | 102.2 | 19.0 | 7.76 | 0.66 | 0.41 | 0.021 | 1.28 | 0.051 |
| MCWM (n=36) | | | 182.1 | 0.72[a] | 1.00 | 1.10 | 103.7 | 18.7 | 7.98 | 0.68 | 0.41 | 0.020 | 1.26 | 0.051 |
| PCWB (n=36) | | | 183.0 | 0.65[b] | 0.95 | 1.16 | 106.1 | 19.5 | 7.90 | 0.67 | 0.43 | 0.022 | 1.23 | 0.050 |
| | ND (n=36) | | 192.5 | 0.76 | 1.01 | 1.14 | 104.8 | 18.6 | 8.65 | 0.70 | 0.43 | 0.021 | 1.30 | 0.055 |
| | HD (n=72) | | 169.5 | 0.67 | 0.97 | 1.13 | 103.2 | 19.6 | 7.10 | 0.64 | 0.40 | 0.020 | 1.21 | 0.046 |
| | | M (n=54) | 192.4 | 0.74 | 1.03 | 1.15 | 107.1 | 19.5 | 7.92 | 0.71 | 0.41 | 0.017 | 1.26 | - |
| | | F (n=54) | 169.6 | 0.69 | 0.96 | 1.13 | 100.8 | 18.6 | 7.83 | 0.63 | 0.42 | 0.024 | - | 0.051 |
| MCWB | ND | M | 213.1 | 0.80 | 1.16 | 1.19 | 114.3 | 20.4 | 9.08 | 0.73 | 0.44 | 0.017 | 1.37 | - |
| | | F | 166.6 | 0.92 | 0.93 | 1.06 | 100.6 | 17.6 | 7.79 | 0.62 | 0.37 | 0.025 | - | 0.052 |
| | HD | M | 169.5 | 0.72 | 1.07 | 1.16 | 98.4 | 20.6 | 7.02 | 0.66 | 0.38 | 0.016 | 1.19 | - |
| | | F | 162.3 | 0.67 | 0.94 | 1.25 | 95.6 | 17.4 | 7.13 | 0.63 | 0.45 | 0.024 | - | 0.050 |
| MCWM | ND | M | 203.3 | 0.83 | 1.07 | 1.16 | 103.7 | 18.0 | 8.97 | 0.76 | 0.45 | 0.017 | 1.31 | - |
| | | F | 181.7 | 0.68 | 1.02 | 1.11 | 102.6 | 19.3 | 8.75 | 0.67 | 0.37 | 0.023 | - | 0.058 |
| | HD | M | 187.4 | 0.73 | 0.95 | 1.14 | 109.8 | 18.4 | 7.40 | 0.70 | 0.42 | 0.019 | 1.21 | - |
| | | F | 156.0 | 0.66 | 0.97 | 0.97 | 98.8 | 19.0 | 6.78 | 0.59 | 0.40 | 0.023 | - | 0.045 |
| PCWB | ND | M | 202.0 | 0.74 | 0.95 | 1.10 | 106.3 | 18.0 | 8.71 | 0.72 | 0.40 | 0.016 | 1.24 | - |
| | | F | 188.3 | 0.59 | 0.96 | 1.26 | 101.5 | 18.2 | 8.60 | 0.67 | 0.53 | 0.029 | - | 0.056 |
| | HD | M | 178.9 | 0.63 | 0.98 | 1.16 | 110.4 | 21.9 | 6.34 | 0.68 | 0.36 | 0.019 | 1.22 | - |
| | | F | 162.7 | 0.64 | 0.92 | 1.12 | 106.1 | 20.1 | 7.94 | 0.61 | 0.41 | 0.023 | - | 0.045 |
| Pooled SEM | | | 5.2 | 0.07 | 0.06 | 0.06 | 3.0 | 0.8 | 0.29 | 0.02 | 0.03 | 0.002 | 0.03 | 0.003 |
| ANOVA | | | p<F | | | | | | | | | | | |
| CT | | | 0.32 | 0.05 | 0.27 | 0.19 | 0.19 | 0.35 | 0.54 | 0.43 | 0.62 | 0.41 | 0.27 | 0.98 |
| CD | | | 0.0001 | 0.05 | 0.22 | 0.73 | 0.34 | 0.04 | 0.0001 | 0.0002 | 0.19 | 0.55 | 0.0001 | 0.002 |
| S | | | 0.0001 | 0.29 | 0.05 | 0.46 | 0.0005 | 0.05 | 0.60 | 0.0001 | 0.41 | 0.0001 | - | - |
| CT×CD | | | 0.87 | 0.40 | 0.68 | 0.13 | 0.002 | 0.02 | 0.59 | 0.46 | 0.05 | 0.54 | 0.02 | 0.29 |
| CT×S | | | 0.20 | 0.35 | 0.11 | 0.15 | 0.67 | 0.004 | 0.004 | 0.44 | 0.007 | 0.25 | - | - |
| CD×S | | | 0.14 | 0.81 | 0.66 | 0.60 | 0.88 | 0.29 | 0.009 | 0.48 | 0.30 | 0.03 | - | - |
| CD×CT×S | | | 0.002 | 0.28 | 0.63 | 0.02 | 0.05 | 0.80 | 0.03 | 0.23 | 0.04 | 0.08 | - | - |

[1] CT = cage type (MCWB = metallic cage with woodchip bedding, MCWM = metallic cage with wire mesh, PCWB = plastic shoebox with woodchip bedding); CD = cage density (ND = 160-cm$^2$/rat, HD = 80-cm$^2$/rat); S = sex (M = male, F = female). Rats were allocated by sex to contain 6 and 12 male/female rats at ND and HD in each of three cage types. Cages were in dimension of 24×40×20 cm, width×depth×height.

[2] Data were obtained at 10 wks of age. BW = body weight; Stom = stomach; SI = small intestine; LI = large intestine; AG = adrenal gland. Values were presented as LSM by cage type, cage density, and sex. Different superscripts within the same columns differ ($p<0.05$) for cage type.

Turkey). Six rats chosen randomly from each group were euthanized by exsanguinations under anesthesia. Then, organs were excised, blotted, and weighed.

### Statistics

Before statistical analyses, blood cell data were normalized by log-transformation. Three-way ANOVA was employed in data analyses using the GLM Procedure (SAS, 1998). The linear model to test the effect of CT, CD, and sex as well as their interactions on BW, organ weights, blood parameters, and hemogram variables was as follows: $Y_{ijkl} = \mu+CT_i+CD_j+S_k+(CT*CD)_{ij}+(CT*S)_{ik}+(CD*S)_{jk}+(CT*CD*S)_{ijk}+e_{ijkl}$, where, $Y_{ijk}$ = response variable, $\mu$ = population mean, $CT_i$ = cage type, $CD_j$ = cage density, $S_k$ = sex, and $e_{ijkl}$ = experimental error. Rat within in the cages was the random term. Moreover, differences among cage types were attained using Tukey's mean comparison option. The effects were considered to be significant at $p<0.05$.

## RESULTS

### Mortality

No death was observed among rats housed at ND, regardless of CT and sex. However, mortality rate at HD was 25, 16.7, and 25% for males and 8.3, 16.7, and 16.7% females housed in MCWB, MCWM and PCWB, respectively, being at a greater rate for males than for females. Most of death cases occurred during the final two weeks of the experimental period. Cause of deaths was not related to any infection. These animals were lighter than their cage mates.

### Growth and organ development

At the end of weaning period (3 wks of age), BW of

**Table 3.** The effects of cage type, cage density, and sex on blood metabolites in growing rats

| Groups[1] | | | Blood metabolites[2] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CT | CD | S | Glucose | TP | Albumin | Creatine | TG | Chol | VLDL | ALP | Ca | P |
| MCWB (n = 36) | | | 223.0$^{a}$ | 5.78 | 3.25 | 0.45 | 60.9$^{a}$ | 52.2$^{b}$ | 18.19 | 346.1$^{b}$ | 11.53$^{a}$ | 7.62$^{a}$ |
| MCWM (n = 36) | | | 202.4$^{b}$ | 5.70 | 3.28 | 0.45 | 69.8$^{a}$ | 56.3$^{a}$ | 16.91 | 394.2$^{a}$ | 11.51$^{a}$ | 7.58$^{a}$ |
| PCWB (n = 36) | | | 202.3$^{b}$ | 5.79 | 3.32 | 0.40 | 42.5$^{b}$ | 56.0$^{a}$ | 17.68 | 386.2$^{a}$ | 11.31$^{b}$ | 7.12$^{b}$ |
| | ND (n = 36) | | 213.9 | 5.72 | 3.30 | 0.41 | 52.9 | 54.1 | 16.73 | 384.8 | 11.41 | 7.36 |
| | HD (n = 72) | | 204.5 | 5.79 | 3.26 | 0.46 | 61.9 | 55.6 | 18.46 | 366.3 | 11.50 | 7.52 |
| | | M (n = 54) | 200.2 | 5.60 | 3.21 | 0.41 | 48.5 | 52.6 | 16.53 | 450.0 | 11.42 | 7.78 |
| | | F (n = 54) | 218.2 | 5.91 | 3.36 | 0.46 | 66.3 | 57.2 | 18.66 | 301.1 | 11.48 | 7.10 |
| MCWB | ND | M | 214.6 | 5.63 | 3.21 | 0.43 | 64.8 | 48.2 | 18.89 | 385.6 | 11.59 | 7.66 |
| | | F | 236.6 | 5.99 | 3.44 | 0.37 | 59.4 | 54.7 | 18.31 | 317.3 | 11.39 | 7.59 |
| | HD | M | 216.4 | 5.39 | 3.00 | 0.56 | 54.7 | 50.5 | 16.12 | 397.1 | 11.46 | 7.86 |
| | | F | 224.3 | 6.11 | 3.33 | 0.46 | 64.8 | 55.5 | 19.44 | 284.4 | 11.70 | 7.37 |
| MCWM | ND | M | 193.7 | 5.58 | 3.18 | 0.27 | 57.3 | 55.2 | 16.38 | 464.0 | 11.30 | 6.83 |
| | | F | 209.7 | 5.77 | 3.35 | 0.53 | 47.0 | 61.3 | 12.17 | 354.7 | 11.70 | 7.63 |
| | HD | M | 202.4 | 5.63 | 3.27 | 0.44 | 65.1 | 56.6 | 19.49 | 491.0 | 11.64 | 8.49 |
| | | F | 203.9 | 5.83 | 3.31 | 0.56 | 109.8 | 52.2 | 19.59 | 267.3 | 11.41 | 7.39 |
| PCWB | ND | M | 211.5 | 5.55 | 3.23 | 0.42 | 24.5 | 47.2 | 13.67 | 550.0 | 11.27 | 7.93 |
| | | F | 217.7 | 5.82 | 3.38 | 0.43 | 64.3 | 58.1 | 20.95 | 237.0 | 11.23 | 6.53 |
| | HD | M | 162.9 | 5.84 | 3.34 | 0.36 | 24.8 | 57.7 | 14.63 | 412.1 | 11.30 | 7.90 |
| | | F | 217.0 | 5.96 | 3.31 | 0.39 | 52.4 | 61.1 | 21.49 | 345.7 | 11.46 | 6.10 |
| Pooled SEM | | | 5.4 | 0.07 | 0.03 | 0.02 | 4.8 | 1.1 | 0.67 | 11.4 | 0.05 | 0.15 |
| ANOVA | | | p<F | | | | | | | | | |
| CT | | | 0.04 | 0.74 | 0.42 | 0.36 | 0.005 | 0.06 | 0.54 | 0.04 | 0.03 | 0.12 |
| CD | | | 0.23 | 0.48 | 0.39 | 0.14 | 0.19 | 0.35 | 0.08 | 0.26 | 0.25 | 0.48 |
| S | | | 0.02 | 0.004 | 0.002 | 0.20 | 0.01 | 0.005 | 0.03 | 0.0001 | 0.44 | 0.002 |
| CT×CD | | | 0.38 | 0.55 | 0.14 | 0.12 | 0.03 | 0.04 | 0.03 | 0.88 | 0.86 | 0.21 |
| CT×S | | | 0.53 | 0.26 | 0.11 | 0.008 | 0.17 | 0.26 | 0.001 | 0.03 | 0.93 | 0.02 |
| CD×S | | | 0.68 | 0.71 | 0.46 | 0.38 | 0.16 | 0.04 | 0.18 | 0.37 | 0.99 | 0.04 |
| CD×CT×S | | | 0.18 | 0.56 | 0.39 | 0.64 | 0.16 | 0.48 | 0.55 | 0.0001 | 0.009 | 0.29 |

[1] CT = cage type (MCWB = metallic cage with woodchip bedding, MCWM = metallic cage with wire mesh, PCWB = plastic shoebox with woodchip bedding); CD = cage density (ND = 160-cm$^2$/rat, HD = 80-cm$^2$/rat); S = sex (M = male, F = female). Rats were allocated by sex to contain 6 and 12 male/female rats at ND and HD in each of three cage types. Cages were in dimension of 24×40×20 cm, width×depth×height.

[2] Data were obtained at 10 wks of age. TP = total protein; TG = triglyceride; Chol = cholesterol; VLDL = very low-density lipoprotein; ALP = alkaline phosphatase. Unit is mg/dl for all metabolites, expect for ALP (U/l). Values were presented as LSM by cage type, cage density, and sex. Different superscripts within the same columns differ (p<0.05) for cage type.

males (46.3±4.3) and females (46.4±4.0) was not different. Table 2 summarizes the effects of CT, CD, and sex on BW and organ weights. CT did not influence the final BW. Doubling CD caused a 12% reduction in BW (p<0.0001). Also, males were 22.8 g heavier than females (p<0.0001). However, there were no two-way interaction effects of CT, CD, and sex on final BW.

Heart weights of rats housed in MCWB and MCWM were similar and both were greater than that in PCWB (p<0.05). Doubling CD was associated with an 11.9% decrease in heart weight (p<0.05). However, there were no sex and two-way interaction effects of main factors on heart weight. CT and CD did not affect lung weight. Males had heavier lung than females (p<0.05).

None of the factors affected stomach weight. Males had a 6.3 cm longer small intestine than females (p<0.0005). Although there were no main effects of CT and CD, doubling CD was associated with 9.7% decrease and 1.1 and 4.1% increases in small intestine of rats housed in MCWB, MCWM, and PCWB, respectively (p<0.002). Rats housed at ND had shorter large intestine than rats housed at HD (p<0.04). Males had longer large intestine than females (p<0.05). Despite a lack of CT effect, magnitude of elongation in large intestine length in response to doubling CD was CT dependent (p<0.02); there were 0.2, 0.3, and 16% increases in large intestine length of rats housed in MCWB, MCWM, and PCWB, respectively, when CD doubled. There was no sex and CD by sex interaction effect on large intestine length, but sex by CT interaction effect (p<0.004). Comparing with females, males had 14.5% longer, 5.3% shorter, and 3.9% longer large intestine in MCWB, MCWM, and PCWB, respectively.

Despite no difference in liver weight due to CT and sex, doubling CD caused reduction in liver weight (17.9%, $p<0.0001$). There was no CT by CD interaction effect on liver weight. However, males and females had different liver weight responses to CT ($p<0.004$) and CD ($p<0.009$). Liver weight for males was 7.3% heavier, 5.2% heavier, and 9.9% lighter than for females housed in MCWB, MCWM, and PCWB, respectively. As CD doubled, liver weight for males and females decreased by 22.4 and 13.1%, respectively.

There was no CT effect on mean kidney weight, but doubling CD was associated with a 8.6% decrease in mean kidney weight ($p<0.0002$). Average kidney weight was greater for males than for females ($p<0.0001$). There were no two-way interaction effects of CT, CD, and sex on kidney weight.

Although there were no effects of main factors, spleen weight in response to different CT varied by CD ($p<0.05$) and sex ($p<0.007$). As CD doubled, spleen weight of rats housed in MCWB, MCWB, and PCWB increased by 2.7 and 0.7% and decreased by 17.1%, respectively. As compared with males, females housed in MCWB, MCWM, and PCWB had 1.3% greater, 11.7% lower, and 22.8% greater spleen weight, respectively. CT and CD did not affect mean adrenal gland weight. Females had a 1.41-fold heavier adrenal gland than males ($p<0.0001$). Moreover, mean adrenal gland weight increased by 8.4% and decreased by 9.7% for males and females, respectively, with doubling CD ($p<0.03$).

CT did not affect mean testis and ovary weights. However, doubling CD resulted in 6.9 and 16.4% reductions in mean testis ($p<0.0001$) and ovary ($p<0.002$) weights, respectively. In response to doubling CD, mean testis weight decreased by 13.4, 7.1, and 1.2% in MCWB, MCWB, and PCWB, respectively. However, alterations in mean ovary weight in response to CD did not differ by CT.

**Blood profile**

Table 3 shows blood chemistry in response to housing conditions and sex. CT but not CD affected glucose concentration; it was the highest for rats housed in MCWB and similar for rats housed in MCBW and PCWB ($p<0.04$). Males had lower glucose concentration than females ($p<0.02$).

There was only sex effect on TP ($p<0.004$) and albumin ($p<0.002$) concentrations: both were lower for males than for females. Despite lacking effects of main factors, creatine concentrations of males and females varied by CT ($p<0.008$). Comparing with females, males had 16.0% greater and 53.5 and 5.9% lower creatine concentration in MCWB, MCWM, and PCWB, respectively.

Rats housed in metallic cages had greater TG concentrations than rats housed in plastic cages ($p<0.005$). Females had a 1.37-fold higher TG concentration than males ($p<0.01$). Despite lacking main effect of CD, TG concentration for rats housed in MCWB, MCWM, and PCWB decreased by 3.8 and 67.8% and increased by 13.1%, respectively, with doubling CD ($p<0.03$). Cholesterol concentrations for rats housed in MCWM and PCWB were similar and tended to be greater than that for rats housed in MCWB ($p<0.06$). Females had greater cholesterol concentration than males ($p<0.005$). Cholesterol concentration of rats housed in different CT and gender varied by CD despite lacking its main effect ($p<0.04$ for both interactions); it increased by 3.1%, decreased by 6.6%, and increased by 12.8% in rats housed in MCWB, MCWM, and PCWB, respectively, with doubling CD. Cholesterol concentration also increased by 9.5% for males, whereas it decreased by 3.1% for females with doubling CD. Males had lower VLDL concentration than females ($p<0.03$). Although there were no main effects of CT and CD, doubling CD caused 4.4% decrease and 36.8 and 4.3% increases in VLDL concentration of rats housed in MCWB, MCWM, and PCWB, respectively. Moreover, comparing with females, males housed in MCBW, MCWM, and PCWB had 7.9% lower, 11.5% higher, and 50% lower VLDL concentration, respectively ($p<0.001$).

ALP activity for rats housed in MCWM and PCWB were similar and both were greater than that for rats housed in MCWB ($p<0.04$). CD did not affect ALP activity. Males had a 1.5-fold greater ALP activity than females ($p<0.0001$). The magnitude of this elevation varied by CT ($p<0.03$); ALP activity for males was 1.3, 1.5, and 1.6-fold greater than for females housed in MCWB, MCWB, and PCWB, respectively. Except for cage type ($p<0.03$), other experimental factors did not affect Ca concentration; rats housed in metallic cages had greater Ca concentration than rats housed in plastic cages. CT and CD did not affect P concentrations. Males had higher P concentration than females ($p<0.002$). Gender responses varied however by CT ($p<0.02$) and CD ($p<0.04$). Comparing with females, males housed in MCWB, MCWM, and PCWB had 3.6, 2.0, and 20.2% greater P concentration, respectively. Moreover, P concentration increased by 8.1% for males, whereas it decreased by 4.1% for females as CD doubled.

**Hemogram**

Hemogram measurements of rats in response to CT, CD, and sex are presented in Table 4. Except for CT ($p<0.01$), other experimental factors did not influence neutrophil count. Rats housed in metallic cages had greater neutrophil count than rats housed in plastic cages. CT did not affect lymphocyte count, but doubling CD caused a 24.2% increase in lymphocyte count ($p<0.002$). There were significant CT by CD ($p<0.04$), CT by sex ($p<0.02$), and CD by sex ($p<0.0002$) interaction effects on lymphocyte

count. Doubling CD caused 0.1% decrease and 49.8 and 26.7% increases in lymphocyte count for rats housed in MCWB, MCWM, and PCWB, respectively. Comparing with females, lymphocyte count for males housed in MCWB, MCWM, and PCWB had 8.9 and 12.9% greater and 30.3% less lymphocyte count, respectively. Lymphocyte count decreased by 4.12% for males, whereas it increased by 61.0% for females as CD doubled. Similar to neutrohil count, ratio of neutrophil to lymphocyte was affected by only CT ($p<0.05$); it was similar for rats housed in metallic cages and greater than for rats housed in plastic cages.

Except for eosinphil count ($p<0.006$), monocyte and basophile counts were independent from the experimental factors. Eosinphil count was the greatest for rats housed in MCWB, and followed by MCWM and PCWB. Because lymphocyte was major leukocyte, the effects of the experimental factors on total leukocyte count were similar to those on lymphocyte count.

There were only CD ($p<0.03$) and sex ($p<0.0001$) effects on erythrocyte count. Doubling CD resulted in a 2.5% increase in erythrocyte count. Moreover, males had a 7.9% greater erythrocyte count than females. Except for sex ($p<0.0001$), other experimental factors did not influence platelet count: males had a 19.9% less platelet count than females. There was only sex effect on hemoglobin concentration ($p<0.0004$); it was 49% greater for males than for females. Hematocrit value for rats tended to be different across CT ($p<0.06$), being greater in rats housed in metallic cages than in plastic cages. Doubling CD was associated with a 2.3% increase in hematocrit value ($p<0.05$). Males had a 5.8% greater hematocrit value than females

**Table 4.** The effects of cage type, cage density, and sex on hemogram in growing rats

| Groups[1] | | | Hemogram variables[2] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CT | CD | S | Neutr $10^3/\mu l$ | Lymph $10^3/\mu l$ | N:L | Monocyt $10^3/\mu l$ | Eosino $10^3/\mu l$ | Basophil $10^3/\mu l$ | Leuko $10^3/\mu l$ | RBC $10^6/\mu l$ | PLT $10^3/\mu l$ | Hb g/dl | HCT % |
| MCWB (n = 36) | | | 0.63[a] | 5.80 | 0.134[a] | 0.014[a] | 0.018[a] | 0.30 | 7.77 | 7.66 | 899 | 14.00 | 39.4[ab] |
| MCWM (n = 36) | | | 0.51[a] | 6.12 | 0.086[a] | 0.017[a] | 0.011[b] | 0.58 | 7.24 | 7.73 | 833 | 14.14 | 40.5[a] |
| PCWB (n = 36) | | | 0.19[b] | 6.28 | 0.036[b] | 0.006[b] | 0.008[b] | 0.18 | 6.67 | 7.58 | 876 | 13.92 | 39.3[b] |
| | ND (n = 36) | | 0.40 | 5.41 | 0.074 | 0.013 | 0.011 | 0.41 | 6.26 | 7.56 | 844 | 13.96 | 39.3 |
| | HD (n = 72) | | 0.49 | 6.72 | 0.097 | 0.012 | 0.014 | 0.30 | 7.53 | 7.75 | 895 | 14.09 | 40.2 |
| | | M (n = 54) | 0.47 | 6.02 | 0.097 | 0.012 | 0.011 | 0.39 | 6.90 | 7.97 | 796 | 14.31 | 41.4 |
| | | F (n = 54) | 0.42 | 6.11 | 0.074 | 0.014 | 0.014 | 0.32 | 6.89 | 7.34 | 944 | 13.74 | 39.0 |
| MCWB | ND | M | 0.67 | 6.60 | 0.099 | 0.011 | 0.019 | 0.68 | 7.98 | 7.81 | 859 | 14.28 | 39.9 |
| | | F | 0.41 | 5.00 | 0.102 | 0.017 | 0.020 | 0.08 | 5.53 | 7.33 | 944 | 13.82 | 37.9 |
| | HD | M | 0.59 | 5.53 | 0.232 | 0.020 | 0.019 | 0.16 | 6.32 | 8.05 | 781 | 14.23 | 41.6 |
| | | F | 0.86 | 6.05 | 0.134 | 0.009 | 0.016 | 0.29 | 7.23 | 7.45 | 1.013 | 13.69 | 38.2 |
| MCWM | ND | M | 0.73 | 5.60 | 0.122 | 0.013 | 0.002 | 1.11 | 7.45 | 7.97 | 737 | 14.40 | 42.1 |
| | | F | 0.33 | 4.20 | 0.072 | 0.027 | 0.013 | 0.38 | 4.95 | 7.28 | 896 | 13.87 | 38.2 |
| | HD | M | 0.43 | 7.48 | 0.063 | 0.013 | 0.011 | 0.04 | 7.98 | 7.99 | 768 | 14.33 | 42.2 |
| | | F | 0.57 | 7.20 | 0.099 | 0.014 | 0.017 | 0.81 | 8.61 | 7.68 | 934 | 13.96 | 39.6 |
| PCWB | ND | M | 0.20 | 6.24 | 0.033 | 0.008 | 0.008 | 0.06 | 6.51 | 7.75 | 765 | 13.77 | 39.9 |
| | | F | 0.09 | 4.85 | 0.018 | 0.002 | 0.005 | 0.18 | 5.12 | 7.24 | 866 | 13.61 | 37.8 |
| | HD | M | 0.20 | 4.67 | 0.063 | 0.003 | 0.007 | 0.30 | 5.19 | 8.25 | 865 | 14.77 | 42.9 |
| | | F | 0.29 | 9.37 | 0.032 | 0.013 | 0.013 | 0.20 | 9.88 | 7.07 | 1,008 | 13.48 | 36.7 |
| Pooled SEM | | | 0.08 | 0.27 | 0.022 | 0.003 | 0.002 | 0.14 | 0.29 | 0.06 | 21 | 0.10 | 0.3 |
| ANOVA | | | ---------- p<F ---------- | | | | | | | | | | |
| CT | | | 0.01 | 0.59 | 0.05 | 0.09 | 0.006 | 0.31 | 0.51 | 0.32 | 0.20 | 0.50 | 0.06 |
| CD | | | 0.48 | 0.002 | 0.48 | 0.78 | 0.32 | 0.60 | 0.004 | 0.03 | 0.10 | 0.39 | 0.05 |
| S | | | 0.72 | 0.82 | 0.48 | 0.61 | 0.23 | 0.75 | 0.97 | 0.0001 | 0.0001 | 0.0004 | 0.0001 |
| CT×CD | | | 0.77 | 0.04 | 0.52 | 0.60 | 0.36 | 0.70 | 0.11 | 0.97 | 0.22 | 0.30 | 0.97 |
| CT×S | | | 0.89 | 0.02 | 0.97 | 0.55 | 0.30 | 0.86 | 0.03 | 0.17 | 0.83 | 0.69 | 0.40 |
| CD×S | | | 0.08 | 0.0002 | 0.95 | 0.58 | 0.96 | 0.12 | 0.0001 | 0.41 | 0.27 | 0.23 | 0.12 |
| CD×CT×S | | | 0.80 | 0.03 | 0.65 | 0.18 | 0.42 | 0.28 | 0.31 | 0.03 | 0.60 | 0.16 | 0.06 |

[1] CT = cage type (MCWB = metallic cage with woodchip bedding, MCWM = metallic cage with wire mesh, PCWB = plastic shoebox with woodchip bedding); CD = cage density (ND = 160-$cm^2$/rat, HD = 80-$cm^2$/rat); S = sex (M = male, F = female). Rats were allocated by sex to contain 6 and 12 male/female rats at ND and HD in each of three cage types. Cages were in dimension of 24×40×20 cm, width ×depth×height.

[2] Data were obtained at 10 wks of age. Neutr = neutrophil; Lymph = lymphocyte; N:L = neutrophil:lymphocyte ratio; Monocyt = monocyte; Eosino = eosinophil; Leuko = leukocyte; RBC = erythrocyte; PLT = platelet cells; Hb = hemoglobin; HCT = hematocrit. Values were presented as LSM by cage type, cage density, and sex. Different superscripts within the same columns differ ($p<0.05$) for cage type.

($p<0.0001$). There were no two-way interaction effects of CT, CD, and sex on hemoglobin concentration and hematocrit value.

## DISCUSSION

### Cage type

Studies dealing with cage material and animal response are limited. Cage material may influence comfort of laboratory animals through affecting basal thermoregulatory process. Following 3,4-methylenedioxymethamphetamine injection, Gordon and Fogelson (1994) showed that basal metabolic rate, evaporative water loss, thermal conductance, and core temperature increased in rats housed in acrylic cages but did not change in rats housed in aluminum cages. It is postulated that cage design with respect to its enrichment influences welfare related parameters through improving physical activity and behavior (Olsson and Dahlborn, 2002; Van de Weerd et al., 2002). Especially mice prefer more complex designed cages to the commonly used cages. However, provision of nesting materials was shown to not affect white and red blood cell counts, hemoglobin concentration, hematocrit value, and internal organ weights (Tsai et al., 2002).

Mering et al. (2001) investigated the effect of flooring system on performance and organ development in Wistar rats. Despite great variation in body fat and adrenal gland weight, BW of rats housed in cages with solid bottom surface and grid floor was not different. Manser et al. (1996) also reported a lack of flooring (solid or grid) effect on feed intake and growth. In a retrospective study, Peace et al. (2001) reported that foot lesions (e.g., ulcers and nodular swellings) were not different among rats housed in cages with wire mesh and in cages made of polycarbonate. Bedding material may lead to increased ammonia concentration and altered temperature and humidity in the cages due to accumulation of feces and urine and excreta related gaseous compounds (Raynor et al., 1983). However, rats prefer particulate bedding materials including sawdust, softwood shavings and paper particles) over wire mesh flooring (Weerd et al., 1996) for dwelling during both resting and activity (Manser et al., 1996).

In the present experiment, cages were made of different materials (aluminum or polycarbonate) and had different flooring systems (wire mesh or woodchip bedding). Mortality rate and BW were not related to CT, but CD and sex. Heart weight for rats housed in plastic cages was lower than for rats housed in metallic cages and appears to be independent flooring system (Table 2). CT did not alter weights of stomach, liver, spleen, kidney, adrenal gland, and genital organs and lengths of intestines. However, lengths of intestines and spleen of rats in different CT varied by CD and sex. Doubling CD and males had greater responses than females in metallic cages, but not plastic cages. Liver weight for males and females responded differently to CT, with greater response in metallic cages than in plastic cages and for males than for females. In these interactions however it is difficult to attribute differences to flooring system. Moreover, magnitude of decline in testis weight was lower in plastic cages than metallic cages. Expect for glucose, lipid related metabolites, ALP activity, and Ca, other blood metabolites were not responsive to CT (Table 3). Blood chemistry data are inconsistent to make inferences about cage material and flooring system. Increases in neutrophil and eosinophil counts in response to different cage material and flooring system are ambiguous (Table 4). However, significant CT by CD and CT by sex interaction effects on lymphocyte count may suggest that males and crowding may increase risk for immune potency in plastic cages. Increased ratio of neutrophil to lymphocyte in rats housed plastic cages may indicate subclinical infections, which may cause stress. Despite not measured, lacking CT effect on erythrocyte count and hemoglobin concentration may indicate that air quality (e.g., $CO_2$, $NH_3$) did not change due to excreta (Memarzadeh et al., 2004). Briefly, our data were inconsistent in terms of justifying CT effects with respect to cage material and flooring system.

### Cage density

Stocking density is perhaps one of the most important aspects of housing conditions influencing well-being of laboratory animals and reliability and quality of biochemical and physiological responses (Anderson et al., 1968; Les, 1968; Serrano, 1971). Increasing CD causes discomfort, limits motor activity, decreases feed intake, and suppresses growth (Rock et al., 1997). Despite no alteration in feed intake and serum TSH concentration, serum insulin and GH concentrations decreased as a result of crowding (Restrepo and Armario, 1989). Gamallo et al. (1986) also reported that crowding cages from 5 to 10 post-weanling rats did not affect feed intake, but it reduced BW and thymus weight and increased adrenal gland and testis weights, suggesting that crowding may delay puberty and that growth-depressing effect of crowding may be related to discomfort, hormonal alteration, and efficacy of nutrient utilization.

Elevated corticosterone concentration and decreased lymphocyte count in response to crowding reflect stress and immune system suppression (Peng et al., 1989; Hayirli et al., 2005a, b). Decreased phagocytic activity, lower release of macrophage colony stimulating factor by spleen, decreased interleukin-1 with greater ability of migration towards chemotactic stimuli, and lower IgM hemagglutination antibody titer to sheep erythrocytes were shown when the number of mice per cage increased from one to five (Salvin et al., 1990). Therefore, decreased phagocytic activity and

superoxide production, lower lymphocyte, greater neutrophil count, and ratio of neutrophil to total leukocyte (Tsukamoto et al., 1994; Stark et al., 2001); elevated plasma corticosterone concentration and decreased natural killer cell activity (Hoffman-Goetz et al., 1992); and reduced production of antibody forming spleen lymphocytes to sheep erythrocytes (Rabin et al., 1987) ascertain immune suppression and stress causing effect of crowding.

Changes in blood metabolites may be linked to organ development and well-being. Weights of liver, kidney, heart and femur by sex in response to crowding from 2 to 5 rats per cage did not differ (Muraoka et al., 1976; Armario et al., 1984a). In another study (Perez et al., 1997), it was shown that prolonging overcrowding however resulted in decrease in plasma glucose and TG but no change in plasma cholesterol concentration. Both rats and chickens have ability of lipogenesis by the liver. Chickens housed in individual cages were shown to have higher liver weight with high TG content than those housed as a group (3 per cage) (Jensen et al., 1976), which could be resulting from limitation of physical activity.

Magnitude of stress in response to crowding varies by sex. Greater level of corticosterone (Brown and Grunberg, 1995) and BW gain (Muraoka et al., 1976) as well as lower adrenal gland weight (Muraoka et al., 1976) for male rats than for female rats could be linked to emotional hyperactivity as reflected by elevated ACTH concentration in response to stressor (Armario et al., 1984a) or feed intake limitation (Armario et al., 1984b). Moreover, as a result of crowding, gonadal function is impaired in male rats (Armario and Lopez-Calderon, 1986).

Crowding resulted in dramatic increase in mortality rate, especially during the last two weeks of the experiment, in this study. Doubling CD also resulted in depressions in BW and organ weights (Table 2). A lack of change in stomach weight may suggest that growth-depressing effect of crowding may not be related to feed intake. However, reductions in weights of lung, liver, and genital organs and length of small intestine may reflect suppressed basal metabolism and nutrient utilization in crowded rats. Increase in adrenal gland weight for males and decrease in adrenal gland weight for females in response to crowding may indicate greater responsiveness to stressors in males than females. Blood chemistry data do not support that crowding may alter partitioning of nutrients or nutrient utilization (Table 3). However, different responses of lipid related metabolites by sex to doubling CD could be related to basal metabolic rate differences. Although there was no alteration in neutrophil to lymphocyte ratio, responsiveness of lymphocyte count to CD may reflect immune suppression resulting from stress as reflected by increased total leukocyte count (Table 4). Similar to decreased heart and lung weights, decreased erythrocyte count in response to doubling CD may be related to increased gaseous accumulation. However, hemoglobin concentration was inconsistent with changes in erythrocyte count in response to doubling CD. Both dramatic increase in mortality rate, BW and organ weight depressions, and lymphocyte and total leukocyte counts due to crowding could be evidence for discomfort and predisposition outbreak of infections.

**Sex**

Animal performance, blood metabolites, and responsiveness to stressors vary by sex. Hurst et al. (1999) reported that males showed more aggressive, competitive, and grooming behaviors than females when CD increased from one to eight rats per cage. It was shown that males had greater ALP activity and glucose and P concentrations and lower albumin and free-fatty acid concentrations than females. There were also no differences in concentrations of Ca and cholesterol (Tsuchiya et al., 1995; Uribe et al., 1995). Hemogram parameters for male and female rats are similar under nonstressing conditions (Robel et al., 1996). Due to behavioral differences under stressing conditions, hemogram parameters however greatly differ by sex (Weisse et al., 1974; Wolford et al., 1987; Uribe et al., 1995).

In the present experiment, higher mortality rate for males than for females could be attributed to competition and aggressive behavior. Males were heavier than females (Table 2). Despite no differences in heart and spleen weights, males had greater lung, liver, kidney weights and longer intestinal length and lighter adrenal gland weight than females. These may indicate that males utilized diet more efficiently than females. Moreover, changes in genital organ weights in response to doubling CD were more pronounceable for females than for males. Especially, liver, adrenal gland, and genital organ weights for males and females differed in response to CD. Most blood metabolites (glucose, TP, albumin, TG, cholesterol, VLDL) were lower and creatine concentration and ALP activity was greater for males than for females (Table 3), which could be related to basal metabolism differences. Lymphocyte count was independent from sex, but it changed differently in response of doubling CD (Table 4). Moreover, males had greater erythrocyte and platelet counts, hemoglobin concentration, and hematocrit value. Different lymphocyte response (decrease in males and increase in females) may ascertain gender differences to overcome stressors (crowding). Nevertheless, despite variability depending upon cage type and cage density, hemogram variables were within ranges of reference values for rats (Leonard and Ruben, 1986; Kahn and Line, 2005), which could be due to sampling after most death cases occurred.

## CONCLUSIONS

In this experiment, physiological variables including growth, organ development, blood parameters, and hemogram measurements in growing rats were evaluated in response to a 3×2×2 arrangement of cage type, cage density, and sex. Cage type effects on growth and organ development and hemogram variables were negligible, but not on blood metabolites. Crowding however had detrimental effects on survival, growth and organ development as well as stress related hemogram variables. The adverse effects of doubling cage density on liver and adrenal gland weights, lymphocyte count varied by cage type and gender, being more pronounceable for male rats than for female rats and for plastic cages than for metallic cages. In general, it appears that rats housed in metallic cages perform better than rats housed in plastic cages and rats housed at density of 160-$cm^2$ per rat than those at density of 80-$cm^2$ per rat. Males also appear to be more responsive to housing conditions than females. Results obtained from the present experiment could be pertinent to further studies dealing with husbandry practices and medical applications.

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