EFFECT OF DOUBLE DENSITY CAGING DURING SPACE SHUTTLE TRANSPORT OF LABORATORY RATS

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INTRODUCTION

The effect of temporary exposure to double density housing in National Aeronautics and Space Administration's (NASA) Animal Enclosure Module (AEM) was determined for a number of physiological and behavioral responses of rats to give scientific inference of animal welfare during transport, and the effects of animal adjustment to the temporary rat density challenge on subsequent research aboard the Space Station Freedom.

This report has six parts. In the first part are general conclusions based on results presented in the five individual reports. More detailed information, covering different aspects of the study, are listed in the five individual reports.

Maghirang, R.G. and G.L. Riskowski. The effect of double density caging during space shuttle transport of laboratory rats: environment.

Sebek, L.A. The effects of double density caging during space shuttle transport of laboratory rats: on food and water intake, weight changes, rat of gain, tissue weights, and physical appearance.

McKee, J.S Effect of double density housing in Mock Animal Enclosure Modules (MAEM) on several physiologic and immunologic responses of male Sprague Dawley rats.

Stricklin, W.R. and H.W. Gonyou. Effect of double density housing in MAEMs on behavioral activity patterns of male Sprague Dawley rats.

Madinduo, T.J. and J. Simon. Gross pathology and histopathology of the gastrointestinal (GIT) and endocrine systems.

TERM DEFINITIONS

AC = acclimation phase.
DC = density challenge phase.
RP = recovery phase.
MAEM = mock animal enclosure module
MAEM-SD = treatment where rats were exposed to MAEMs at standard density during the DC. Rats were housed in polycarbonate cages during the AC and RP.
MAEM-DD = treatment where rats were exposed to MAEMs at double density during the DC. Rats were housed in polycarbonate cages during the AC and RP.
PC = treatment where rats were exposed only to polycarbonate cages.

PROJECT DESCRIPTION

Male Sprague Dawley rats were housed in groups of four in polycarbonate cages at recommended density and thermal environmental conditions (NIH, 1985) for 14 days prior to testing to ensure uniform acclimation to those conditions. Body weights averaged 286 $\pm$ 7 g at the end of acclimation. Rat cages were assigned randomly to three treatments: 1) 4 rats/polycarbonate cage (877 cm^2, 20.3 cm high, 220 cm^2/rat), 2) 4 rats/mock AEM (MAEM) (620 cm^2, 155 cm^2/rat), and 3) 8 rats/MAEM (620 cm^2, 77.5 cm^2/rat). A comparison between the MAEM-DD and MAEM-SD treatments was done to determine if doubling rat density in AEMs stressed the rats. A comparison among MAEM treatments and the PC treatment was done to determine if any stress indications were due to the AEM.

During this density challenge phase, all treatments were maintained at the same thermal environmental conditions (22.5°C & 50% RH) for 10 days. After the density challenge phase, half the rats from each group were sacrificed for body tissue and fluid analyses. The remaining half of the rats were housed at a density of 4 rats/cage in polycarbonate cages at normal thermal environmental conditions for an additional 10 days to determine if there were any differences in responses between treatments after a recovery period. The remaining rats were examined and sacrificed for body tissue and fluid analyses at the end of the recovery phase.

The photo period was 12 h light and 12 h dark (on at 0700 h). Each rat received NASA food bars and water ad libitum throughout the study. There were a total of five replications of each treatment. There were eight rats per treatment per replication so there were 40 rats per treatment over the five replications. All treatments were represented during each replication (Table 1).

Table 1. Example of one replication sequence of the experiment.

Phase:	Acclimation	Density Challenge	Recovery
Duration:	14 days	10 days	10 days
Treatment	Number of Rats and Cage Type		
4 Rats/PC	4 PC	4 PC (sacrificed)	
4 Rats/PC	4 PC	4 PC	4 PC (sacrificed)
4 Rats/MAEM	4 PC	4 MAEM (sacrificed)	
4 Rats/MAEM	4 PC	4 MAEM	4 PC (sacrificed)
8 Rats/MAEM	4 PC & 4 PC	8 MAEM (4 sacrificed)	4 PC (sacrificed)

Note: PC = polycarbonate cage; MAEM = mock animal enclosure module.

Air temperature, relative humidity, air velocity, light intensity, noise level, and ammonia level were monitored in the room daily throughout the acclimation period. The room was a standard laboratory animal room located in the University of Illinois' Plant and

Animal Biotechnology Laboratory (PABL) which has several laboratory rooms and laboratory animal care certified personnel who managed the rats according to NIH guidelines (NIH, 1985). The room was ventilated at a rate of 12 air changes per hour with 100% outside air (i.e., no recirculation air).

The PC dimensions were 203 mm base width by 432 mm length by 203 mm height standard polycarbonate cages with solid sides and floor. The top was wire grid that held the food pellets and Lixit waterer. The PCs had heat treated hardwood shavings on the floor for bedding. A Lixit waterer was placed near the cage center about 90 mm above the floor at an angle of approximately 45°.

Three MAEMs were constructed to simulate the AEM used by NASA for transport of rats in the space shuttle, Figures 1 and 2. The MAEMs had the same interior dimensions and materials as the AEMs. This included the space occupied by the water storage tank and waterers in the center of the AEMs. The MAEMs were placed in the vertical position as they would be in the middeck locker of a space shuttle. Rat wastes collected on a pan below the cage which were removed and cleaned at the end of the 10 day density challenge period. The MAEMs were constructed to provide a uniform distribution of airflow (0.13 m/s; 25 fpm velocity) approaching the rat cages. An air velocity of 0.13 m/s was chosen to ensure removal of rat wastes from the AEM on a timely basis when under microgravity conditions.

ENVIRONMENTAL INDICES

During both the density challenge and recovery phases, the following environmental indices were monitored:

1. Room air temperature (every 15 min)
2. Air temperature within each cage (every 15 min)
3. Room air relative humidity (two times daily, 0800 h and 2000 h, with psychrometer)
4. Air velocity magnitude and direction approaching the top of each cage (twice daily, 0800 h and 2000 h)
5. Light intensity at the top center of each cage (twice daily, 0800 h and 2000 h)
6. Noise level at the top center of each cage (twice daily, 0800 h and 2000 h)
7. Ammonia level at the center of the room (once daily, 0800 h)
8. Ammonia level from within each cage (5th and 10th day, 1200 h, for both phases)
9. Static pressure across the rats in MAEMs (twice daily, 0800 h and 2000 h)

Room air temperature and relative humidity also were monitored continuously with a recording hygrothermograph.

The overall mean room air temperature was 22.2°C ($\pm$0.1°C) and relative humidity was 54% ($\pm$1%). Cage and MAEM air temperatures were slightly higher than the room air temperature because of heat generated by the rats, Figure 3. The MAEM-DD treatment had higher temperatures (approximately 1°C) than the PC or MAEM-SD treatments due to the higher number of rats, but the difference was small and would have had little effect on the rats.

Heat loss from the rats in the MAEMs was estimated from the difference in air temperature entering and exiting the cage area as described in the report by Maghirang and Riskowski, Figure 4. Single density rats had a consistently higher heat loss on a per rat basis than the double density rats. The higher density rats had closer contact due to the extra rats, which would have reduced heat loss to the ambient air.

The mean air velocity approaching the top of the PCs varied from 0.07 to 0.18 m/s; whereas, the mean air velocity in the MAEMs ranged from 0.12 to 0.14 m/s, Figure 5. Air velocity within the PCs would be much lower than the approach air velocity; whereas, the air velocity within the MAEM cages would have been essentially the same as the approach air velocity.

Mean noise levels within the PCs and MAEMs ranged from 50 to 65 dBA. Overall mean noise levels were uniform across treatments, Figure 6.

Light levels at the top of the PCs ranged from 30 to 68 lux during "light" hours and from 4 to 11 lux during "dark" hours. Light levels at the sides of the MAEMS was 200-251 lux during "light" hours and 3-4 lux during "dark" hours. There was a large difference between the MAEMs and PCs; however, the light levels within the cages near the rats were much closer. The light levels within the PCs were slightly above 80 lux (Figure 7) and within the MAEMs were in the mid-60 lux range during "light" hours.

Ammonia levels and static pressures across rats were negligible.

RAT RESPONSES MEASURED

The following data were collected for both the density challenge and recovery phases:

1. Weights of food and water consumed by each group of 4 or 8 rats during both phases
2. Rat body weights before and after both phases
3. General physical appearance (coat, eyes, ears) after each phase
4. Assessment of animal behavior during both phases
5. Lectin-induced lymphocyte proliferation for half the rats after each phase
6. Corticosterone levels for half the rats after each phase

7. Total plasma proteins, differential leukocyte counts, and hematocrit for half the rats after each phase
8. Gross and histologic tissue evaluation of the gastrointestinal and endocrine organs for half the rats after each phase

Feed was weighed to the nearest 0.1 g prior to being placed in a cage before each phase and again at the end of a phase. In addition, feed was weighed at day 2 and day 5 during the DC and at day 5 during RP. Water intake was measured daily to the nearest 0.5 ml. Feed and water intakes were calculated on a cage mean basis. Rats were weighed individually to the nearest 0.1 g at the beginning and end of each phase. Rats were weighed at day 2 and day 5 during the DC phase and at day 5 during the RP phase. Percent body weight change was calculated based on a cage mean. Feed conversion efficiency was calculated by dividing the mean daily weight gain per rat by the mean daily feed intake per rat for each phase.

Physical appearance of the rats was evaluated at the end of the DC and RP phases. Rats which were to be sacrificed after the DC and RP phases were first evaluated on a cage basis. Cages were ranked from best to worst based on the overall appearance of hair coat, eyes, ears, and nose. At the end of the DC phase, the rats that were to go on to the RP phase were evaluated individually for the same criteria. The rats that were ranked individually were placed in separate cages and ranked from best to worst for the condition of hair coat first, ears second, and finally eyes and nose. Judgements of coat condition was based on color and degree of mattedness, ears were based on cleanliness and color, and eyes and nose were based on color and the appearance of any discharges.

During the DC phase, one cage from each treatment was video taped for the first 48 hours and again on day 9 for 24 hours. For the PC and MAEM-SD cages one camera was positioned to take pictures from one end of the cage. Two cameras were used for the MAEM-DD (front and back) since it was difficult to monitor rat behavior at the higher density with just one camera. Black and white low light cameras with 0.5 lux minimum scene illumination were used. During the RP phase, all cages were videotaped for 24 hours on days 2 and 9. The camera signals were channeled through a switcher that changed the picture from cage to cage every 60 seconds during both phases.

The video tapes from days 2 and 9 of both phases were viewed to determine time budgets of: 1) standing, 2) standing erect on hind legs, 3) sitting, 4) lying, 5) eating, 6) drinking, 7) sleeping, 8) grooming, 9) playing, and 10) licking. Negative behaviors, such as fighting, would have been noted, but none was detected in this study. The video tapes were scan sampled at one minute intervals for the first 20 minutes of each hour of the 24 hour period. Because the pictures were switched among 4 cages, each cage was scanned approximately five times in that 20 minute period. The huddling index (HI) was calculated four times each hour for each group and then pooled for each 4-hour period.

HI = (Number of rats lying in contact with others) / (Number of rats in group)

Rats were considered to be huddling when two or more rats were lying with some part of their body in contact with another rat's body. With four rats to a cage the percentage of huddling could be 0%, 50%, 75%, or 100%. The area of floor not covered by rats was estimated.

At the end of the DC and RP phases, four rats from each treatment were sacrificed for blood and tissue analyses. To minimize stress reactions from euthanasia, the rats were anesthetized with carbon dioxide by lowering the entire cage of rats into a container and then charging the container with CO_2. The rats were guillotined and blood samples were collected.

Corticosterone (the major rat glucocorticoid) concentrations were evaluated from plasma obtained during sacrifice. Values were determined by using the RSL ^{125}I Corticosterone Kit for rats and mice (ICN Biomedicals Inc., Costa Mesa, Ca). Radioactivity was measured with a Beckman gamma counter. Circulating corticosterone was measured because it is a classic indicator of stress. To avoid the circadian rhythm of corticosterone release, with the highest concentrations being reported between 1600 and 2200, blood samples were collected at 0900. After performing preliminary work on the effects of different euthanasia procedures on corticosterone values, it was discovered that using CO_2 anesthesia prior to decapitation provided values closest to baseline values thereby reducing any false readings as a result of any extraneous stressors such as handling. Handling variables were reduced since rats were anesthetized in their respective cages.

Lectin-induced lymphocyte proliferation was evaluated because it is negatively correlated to circulating corticosterone levels. Cell suspensions prepared from spleens and erythrocytes were lysed with 0.83% NH_4Cl in 0.1% $KHCO_3$/0.01 mM EDTA. Splenocytes were suspended at 5×10^6 cells/mL in RPMI 1640 medium supplemented with 5% fetal calf serum, 100 units of penicillin/mL, 100 μg streptomycin/mL and 24 mM $NaHCO_3$. Aliquots consisting of 100 μL of cells and 100 μL of various concentrations of Concanavalin A (Con. A) were plated out in 96-well plates (each sample was done in triplicate) and incubated at 39°C in 7% CO_2 for 72 hours. One μCi of [methyl-3H]thymidine was added to each well and incubated for an additional 18 hours. Cells were harvested onto glass fiber disks using a multiple cell harvester. The disks were dried with 70% ethanol and placed in an 80°C oven for 15 minutes. Scintillation cocktail of toluene-Omniflour was added and radioactivity was measured with a Beckman LS 5801 liquid scintillation counter.

Total plasma protein was evaluated from plasma obtained during sacrifice. Values were determined by using a Total Protein assay (Sigma Diagnostics, St. Louis MO).

For differential leukocyte counts, blood smears were made at the time of sacrifice, stained and analyzed with special attention to neutrophils and lymphocytes. The neutrophil:lymphocyte ratio is a classic indicator of stress and is correlated positively with circulating corticosterone levels.

Packed cell volume for each rat was obtained as the mean of three replicate hematocrit readings.

For gross tissue evaluation, rats were necropsied and adrenals, thymus, and testes were removed, trimmed of excess tissue, weighed, and placed in formalin. The GI tract was tied at the esophageal opening to the stomach and the colon rectal area and removed. The GI tract and contents were weighed, flushed, weighed again (empty), and preserved in formalin. The tissue weights were calculated as a percentage of total body weight and means were calculated on a per cage basis. Gross evaluations of GI mucosa from the stomach, duodenum, and ileum were made, with special notice for evidence of ulcerations and the occurrence of circumscribed distentions on the serosal surface.

For histologic evaluation of the GI tract and endocrine tissue, samples of gastric stomach, duodenum, ileum, large intestine, adrenals, testis, thymus, and the right femur were removed from each sacrificed rat and fixed in 10% buffered formalin solution. The bone tissue (femur) was decalcified in a solution of formic acid and sodium nitrate mixture before processing and staining. All tissues were processed at the Veterinary Histopathology Laboratory of the University of Illinois at Urbana-Champaign, and stained with standard hematoxylin-eosin. Tissue samples were examined microscopically for histologic evaluation. The evaluators were aware of the general treatments in this study but did not know which tissue belonged to which treatment.

Since some circumscribed distentions on the serosal surface and histopathologic effects were observed on the gastrointestinal tracts of experimental rats in replications 1 and 2, four non-trial comparison rats that had been fed high-fiber chow diets were compared with the experimental rats which were fed only NASA food bars. This small preliminary study was conducted to determine if there was any evidence that the NASA food bars may be causing the observed abnormalities. Two of the non-trial comparison rats (rats 1 and 2) were first fed the NASA food bars for two weeks during an acclimation phase and then fed a high-fiber chow diet for 10 days (DC phase). The other two non-trial comparison rats (rats 3 and 4) were fed only the high-fiber chow diet. All high-fiber chow fed rats were housed in PCs during both acclimation and DC phases and were sacrificed for analysis after the DC phase. Comparisons were made on the gross pathologic and microscopic examinations of the gastrointestinal tract.

A general linear models procedure, using the statistical analyses system (SAS Institute, 1990), was performed on all but the gross and histologic work to determine if any treatment and replication differences were present. Differences between treatment and replication means were analyzed with the general linear models (GLM) procedure of SAS. Each cage of rats was an experimental unit. Thus, rats within a cage contributed to the mean of the cage, which was used as one data point in calculating treatment means. A randomized complete block analyses was used to investigate the effect of housing on body weight, tissue weights, and immunologic and physiologic responses of rats. The model included replication as blocks, housing systems as treatments and replication x treatment; replication x treatment served as the error term.

RESULTS

Food and water consumption

Rats generally consumed a similar amount of food throughout all treatments and all phases (Table 2). During the DC phase, the MAEM-SD rats consumed slightly more ($P<0.05$) food than the rats in the other two treatments. Food consumption values included any unretrieved food spillage. During the DC phase, rats in the MAEM treatments may have lost more food through the wire floors than PC rats on solid floors. Originally, we hoped to retrieve and weigh-back any food spillage, but the small food particle size mixed in with the feces and urine made this task impossible. There were no statistical differences ($P>0.05$) in food consumption among treatments during the RP phase.

Table 2. Food and Water Consumption Means

Comparisons of treatment means were made separately for DC and RP phases. No comparisons were made between DC and RP values. Means with different superscripts differ ($P<0.05$); means without superscripts do not differ. SEMs are given in Table 1 of report by Sebek.

Phase:	AC	DC			RP		
Treatment:		MAEM-DD	MAEM-SD	PC	MAEM-DD	MAEM-SD	PC
Food (g/rat/day):	23.4	24.6^{a}	27.1^{b}	23.6^{a}	25.4	26.1	26.5
Water (ml/rat/day):	24.9	43.2^{c}	37.9^{c}	25.4^{d}	24.9	26.4	26.8
Normal food and water consumption*:							
Food (g/rat/day):	24.0	30.4	30.4	30.8	33.8	33.6	34.6
Water (ml/rat/day):	24.0-28.8	30.4-36.4	30.4-36.5	30.8-36.9	33.8-40.6	33.6-40.3	34.6-41.5

*For Sprague-Dawley male rats at the average weight of the experimental rats in the corresponding treatment and phase with standard rat-chow diet. Normal values are from Harlan Sprague Dawley, Inc.

MAEM-SD and MAEM-DD rats consumed 50-70% ($P<0.05$) more water than PC rats. This may have been due to higher water spillage in the MAEMs. MAEMs have four Lixit waterers that are close to the floor area at a location where the rats preferred to gather. Consequently, rats may have incidentally pushed against the Lixits in the MAEMs. Lixits were located higher in the PCs. There were no statistical differences ($P>0.05$) among treatments during the RP phase.

Body weight change and feed conversion efficiency

Body weights averaged 286 $\pm$ 7 g at the end of acclimation. As shown in Table 3 and Figure 8, MAEM-DD rats had a lower weight ($P<0.05$) increase than PC rats during the DC phase, but the MAEM-SD rats did not differ ($P>0.05$) from the MAEM-DD or the PC rats. MAEM-DD and MAEM-SD rats expressed a compensatory growth during the RP phase and had a slightly higher percent body weight change from the start of DC to the end of RP than the PC rats, although the difference was not significant ($P>0.05$).

The rats in this study had a growth rate apparently the same as that which is normal for male Sprague-Dawley rats, Figure 9. Male Sprague-Dawley rats do not normally reach the upper portion of the "S" growth curve at an age of 82 days which corresponds to the rat age at the end of the RP, Figure 9.

During the DC phase, the means of the weight gain/food consumption ratios (food conversion efficiencies) were lower for the MAEM-DD and MAEM-SD treatments than for the PC treatment ($P<0.05$). The lower food conversion efficiency of the rats in the MAEM treatments during the DC phase may have been due to food particle spillage through the wire floors. Rats in the PC cages at least had the opportunity to retrieve any spilled food while the MAEM rats did not. During the RP phase, there was no statistical difference ($P>0.05$) between treatment means. However, the MAEM-DD and MAEM-SD rats had an increase in food conversion efficiency in the RP phase relative to the DC phase.

Table 3. Rat Body Weight Increase and Food Conversion Efficiency.
First numbers are weight gain where units = percent gain from rat weight at start of phase; numbers in ( ) = weight gain/feed consumption ratios. Statistical comparisons of the treatment means were made separately for DC and RP phases. No comparisons were made between the DC and RP values. Means with different superscripts differ ($P<0.05$); means without superscripts do not differ.

Treatment	Phase			
	DC		RP	
MAEM-DD	$10.7\pm0.91\%^{a}$	(0.125 ± 0.010^{c})	$13.2\pm1.26\%$	(0.163 ± 0.013)
MAEM-SD	$12.8\pm2.28\%^{ab}$	(0.134 ± 0.019^{c})	$13.2\pm2.74\%$	(0.150 ± 0.019)
PC	$15.8\pm0.94\%^{b}$	(0.190 ± 0.010^{d})	$10.0\pm0.85\%$	(0.154 ± 0.031)

Physical appearance

During the DC phase, the individual ranking analysis showed the PC rats had the best physical appearance, followed by MAEM-DD rats, and finally MAEM-SD rats ($P<0.05$). During the DC phase, the cage rankings showed PC cages having the best physical appearance, with MAEM-SD rats second, and MAEM-DD last ($P<0.05$). During the RP phase, no differences ($P>0.05$) in physical appearance were found between the treatment cages. Density and cage type have a detrimental effect on physical appearance, but the rats recovered within 10 days after being placed in a standard PC cage at standard density. The lower appearance rankings for rats in the MAEMs may have been due to leakage from the low waterers in those cages.

Behavioral Activity Patterns

The levels of major activities (lying, sitting, standing normal, and standing erect) by cage type and phase of study are presented in Figures 10-13. Analysis of eating, drinking, sleeping, grooming, and playing behavior did not show patterns that differed between treatments. A split split-plot analyses was used to determine levels of significance for main effects (Table 4). Of the major activities, the phase by cage-type interaction was significant for only lying frequency.

During DC, MAEM-DD rats spent more time ($P<0.05$) lying than during the other treatments (Figure 10). This greater lying time was reflected in numerically less standing in the normal position and less sitting, which both approached significance ($P<0.10$). Erect standing did not show any trend across cages type or phase. During the RP phase, the lying time of the MAEM-DD rats decreased to be essentially the same as the other treatments.

Table 4. Mean Squares and levels of significance for effects as determined by Split Split-Plot Analysis.

Source	Degrees of Freedom	Mean Squares			
		Lying	Sitting	Stand-norm	Stand-erect
Treatment (T)	2	422.4[a]	93.8	74.4	47.0
R*T (Error 1)	8	60.2	82.4	47.3	13.4
Phase (P)	1	1124.3[a]	239.1[a]	373.3[a]	1.6
T*P	2	502.4[a]	73.6	86.7	26.5
R*T*P(Error 2)	8	105.4	26.8	37.8	16.0
Hour(Day)	10	6281.8[c]	1578.6[c]	248.1[c]	635.5[c]
P*T*Hour	25	189.3[b]	50.1	28.0	20.4
Remainder	269	100.3	35.1	16.5	24.3

[a] $P<0.05$.
[b] $P<0.01$.
[c] $P<0.001$.

Figures 14-16 show similar patterns of lying behavior through the day for all treatments and phases; however, the MAEM-DD rats displayed more lying during the dark periods of the DC phase ($P<0.05$; Table 4). During DC, the MAEM-DD rats showed a generally flatter diurnal pattern (especially at 1900 hours) of lying activity than did PC and MAEM-SD rats during DC. During RP, diurnal activity of MAEM-DD rats returned to a diurnal pattern similar to the other groups.

During DC, the MAEM-DD rats had a higher huddling index (Figure 17) which may have been due to the higher density forcing rats into closer proximity. There was less free floor area for the MAEM-DD rats during the double-density challenge phase (Figure 18) which may have been due primarily to the larger number of rats. However, the MAEM-DD rats occupied more floor area on a per rat basis than the lower density rats which could indicate that double density rats may have been warmer from the additional body heat.

Drinking, eating and play behavior did not show a pattern across the cage types and phases. The MAEM-DD rats engaged in 3.1 licks/minute/rat versus 4.6 and 4.8 for the MAEM-SD and PC rats, respectively (Figure 19). During RP, the rates of licking did not significantly differ among the treatments. Licking is possibly a displacement activity resulting from a thwarting of another behavioral drive, or it can be used for evaporative cooling when rats are heat stressed. Lower licking rates can also lead to lower appearance rankings as was found for the MAEM-DD rats.

Double density housing of rats resulted in between 5 and 10% more time spent lying. This decreased activity was reflected in less sitting and less normal standing. The amount of standing erect was not affected by doubling the density. The amount of licking appeared to decrease in double density housing. Other than decreased rates of activity, none of these behavioral differences were obvious indicators of a state of negative well-being. All levels of activity returned to normal after density was decreased.

Lectin-induced lymphocyte proliferation

MAEM-DD rats generally had a higher degree of proliferation than the MAEM-SD and PC rats at all concentrations of mitogen during the DC phase (Figure 20a). During the RP phase, MAEM-DD rats had proliferation similar to PC rats at all concentrations of mitogen, while the MAEM-SD treatment had slightly higher values, Figure 20b. However, differences ($P > 0.05$) among treatments were not statistically significant ($P > 0.05$) for either phase.

Plasma Corticosterone

MAEM-DD rats had a 54% and 61% increase in mean plasma corticosterone levels during the DC phase as compared with the MAEM-SD and PC rats, respectively (Table 5 and Figure 21). However, because of the large standard error, none of the differences were significant ($P > 0.05$). Also, none of the treatment plasma corticosterone levels were abnormally high relative to other rats exposed to known stressors in other studies (Gamallo et al., 1986, Raab et al., 1986; Hoffman-Goetz et al., 1992).

Table 5. Circulating Plasma Corticosterone.
Units = ng/mL. Comparisons of the treatment means were made separately for the DC and RP phases. No comparisons were made between the DC and RP values. Means without different superscripts do not differ (P>0.05).

Treatment	Phase	
	DC	RP
MAEM-DD	45.2$\pm$18.3	25.6$\pm$ 7.9
MAEM-SD	29.3$\pm$10.3	19.0$\pm$ 3.0
PC	28.1$\pm$ 9.5	37.2$\pm$14.6

Individual rats within the treatments did indicate the presence of stress by exhibiting increased plasma corticosterone levels and neutrophil:lymphocyte ratios. Only eight rats (out of 113 total) expressed high corticosterone levels exceeding 100 ng/mL (depending on the reference, levels over 100 ng/mL are still considered within baseline range). Four of these rats were from MAEM-DD, one from MAEM-SD, and three from PC. However, only 50% of those eight rats expressed a corresponding increase in neutrophil:lymphocyte ratios which would be expected. Therefore, the high corticosterone levels exhibited by the other 50%, may have been due to their response to the euthanasia procedure, instead of treatment, because the corticosterone did not have time to influence the leukocytes within the vasculature.

During the RP phase, plasma corticosterone for the MAEM-DD rats decreased 43%, but levels in the MAEM-SD also decreased (35%) so MAEM-DD rats still had higher levels than MAEM-SD rats. Plasma corticosterone levels in PC rats showed an increase during the RP phase, but this increase could be attributed to two rats that had abnormally high corticosterone levels relative to the others in the same treatment. If those two data points were removed, a new mean for PC during RP would be 27.2 ng/mL rather than 37.2 ng/mL, which would be about the same level found for the PC rats during DC. There were no differences (P>0.05) among RP means. Therefore, although double housing density may have increased plasma corticosterone levels, levels were not abnormally high plus the rats recovered to levels of unchallenged rats within 10 days after the density challenge.

Total plasma proteins, blood differentials, and hematocrit

During the DC phase, PC rats had the highest level of plasma protein while MAEM-SD rats had the highest during the RP phase, Figure 22. Both MAEM-DD and PC rats showed a decrease at the end of RP while MAEM-SD showed an increase. However, there were no statistical differences (P>0.05) among treatments for plasma protein.

The only statistical difference (P<0.05) between cell types was the percent of monocytes between MAEM-DD and PC during the RP phase. Due to the overall small numbers of monocytes counted, no treatment effect is attributed to this difference.

Since there were no differences in plasma corticosterone levels between treatments, it would follow that there would be no differences in the neutrophil:lymphocyte ratios and none were found. Individual rats which expressed high plasma corticosterone levels exhibited a higher neutrophil:lymphocyte ratio compared to rats with low levels of corticosterone. Calculating ratios by cage means masked these individual differences.

Both MAEM treatments had slightly higher packed cell volumes (PCV) than the PC treatment during both phases, Figure 23. MAEM-DD had a slightly higher PCV during DC while MAEM-SD increased above MAEM-DD during RP. However, there were no differences ($P > 0.05$) in treatments for PCV.

Gross and histopathology of the GIT and endocrine systems

Tissue weights as a percent of body weight are presented in Table 6. Of the tissue weights, only the adrenal glands had statistical differences ($P < 0.05$) between the MAEM treatments and the PC treatment. During DC, the MAEM adrenal weights were slightly higher than the PC weights, but during RP the MAEM adrenal weights were slightly lower than the PC weights.

Gross evaluation of the GI mucosa from the stomach, duodenum, and ilium of all rats revealed no evidence of ulceration developed during the investigation across treatments. Gross pathologic examination revealed the occurrence of circumscribed distentions on the serosal surface, along the gastrointestinal tract, except on the surface of the duodenum, of all the rats, regardless of treatment. Mean distention counts were lower for rats in DC than RP. Rectal hemorrhages and hard pelleted stools were observed in some of the rats, independent of housing. These observations indicate an irritation in the GI tract which may be an inherent problem to the rats or problems with the food bars.

The four high-fiber chow fed rats had fewer distentions, and larger and longer gastrointestinal tracts (about twice the length) than the NASA food bar fed rats. Since distentions were observed in all rats, other inherent factors may be responsible. Further studies may be needed to determine the effects of the food bar diet on the gastrointestinal tract of the rat.

Table 6. Body Tissue Weights.
Tissue weights are presented as a percent of body weight.

	Treatment		
	MAEM-DD	MAEM-SD	PC
DC:			
GI tract (empty)	3.51 ± 0.12	3.76 ± 0.08	3.62 ± 0.06
Testes	1.07 ± 0.03	1.11 ± 0.03	1.08 ± 0.03
Thymus	0.20 ± 0.16	0.20 ± 0.009	0.21 ± 0.009
Adrenals	0.016 ± 0.002[a]	0.018 ± 0.002[a]	0.014 ± 0.002[b]
RP:			
GI tract (empty)	3.30 ± 0.03	3.42 ± 0.07	3.39 ± 0.05
Testes	0.99 ± 0.02	0.98 ± 0.05	1.01 ± 0.01
Thymus	0.16 ± 0.005	0.17 ± 0.005	0.16 ± 0.008
Adrenals	0.012 ± 0.0007[c]	0.012 ± 0.0003[c]	0.014 ± 0.0006[d]

[a,b]Means in the same row with different superscripts differ ($P > 0.005$).
[c,d]Means in the same row with different superscripts differ ($P < 0.05$).
Rows without superscripts are not significantly different ($P > 0.05$).

Microscopic examination of the stomach revealed mild catarrhal inflammation of the glandular stomach for most rats. The ileal histology showed mild infiltration of lymphocytic cells in most rats. Several rats had prominent Peyer's Patches and, in addition, some of these developed mild catarrh. In some cases the ileal surfaces revealed hyperplastic lymphocytic elements in the mucosa. Some of the Peyer's Patches in this area were confluent to a degree suggestive of lymphosarcoma, which in some cases nearly penetrated the serosal wall and contained areas of hyperplasia foci suggestive of the so-called starry sky "appearance". The Peyer's Patches were hyperplastic, and confluent and, in certain cases, they developed lymphoid aggregates which were essentially malignant, as evidenced by hyperchromasia, variation in cell size, and peripheral extension. In some cases, the patches were confluent and hyperplastic and nearly extended through the serosal wall of the ileum, changes suggestive of early neoplasia. The endocrine tissues, including the testis, thymus, and adrenals, were normal, as were the bone and bone marrow.

CONCLUSIONS

For the DC phase, the only statistically significant differences ($P < 0.05$) found among treatments were:

MAEM-SD rats consumed more food than the other two treatments but results would be affected by the inability to retrieve spilled feed in the MAEMs.

MAEM-SD and MAEM-DD rats used more water than PC rats. This difference may be attributed to water spillage that is a result of the location of the Lixit waterers in the MAEMs.

MAEM-DD rats had a lower percent body weight increase than the PC rats.

MAEM-SD and MAEM-DD rats had a lower food conversion efficiency than PC rats.

MAEM-SD rats had the worst individual ranking for physical appearance, then MAEM-DD rats, and PC rats had the best. MAEM-DD rats had the worst cage ranking for physical appearance, then MAEM-SD rats, and PC rats had the best.

MAEM-DD rats spent more time lying and had lower licking rates than the MAEM-SD or PC rats.

MAEM-DD and MAEM-SD rats had higher adrenal weights than the PC rats.

The MAEM-DD rats had noticeably higher levels of circulating plasma corticosterone than the MAEM-SD and PC rats, but the difference was not significant ($P>0.05$) because of the large standard error. Levels of plasma corticosterone displayed by the MAEM-DD rats during the DC phase were not abnormally high.

During the RP phase, the only statistical difference ($P<0.05$) found between treatments was the percent of monocytes between MAEM-DD and PC, and the MAEM-DD and MAEM-SD rats had lower adrenal weights than the PC rats. However, due to the overall small counts of monocytes counted, no treatment effect was attributed to the difference in monocytes. Since the MAEM adrenal weights were lower during RP, it may indicate some compensatory reaction for recovery.

Since many of the significant differences were between the two MAEM treatments and the PC treatment, there may be some stress due to the AEM housing relative to the PC housing. However, stress indicators did not appear to be abnormally high and the rats appear to recover from any housing stress within 10 days after being placed in PC housing.

The statistically significant evidence that the double density housing (MAEM-DD) was more stressful than the single density housing (MAEM-SD) was that the MAEM-DD rats had lower percent body weight gain, the MAEM-DD rats ranked worst for cage ranking of physical appearance (but not for individual ranking), and the MAEM-DD rats spent more time lying and had lower licking rates. The plasma corticosterone level was greater for the double density, but the differences were not statistically significant ($P>0.05$). Consequently, there was little or no stress increase due to double density housing. If there was any increase in stress due to double density housing, the rats recovered within 10 days after the density challenge.

Note: The observed water leakage problem in the MAEMs may also be a problem in the actual animal enclosure modules. As such, the AEM design may have to be modified to minimize this problem. Possible modifications include increasing clearance between floor and lixit. Another is moving the water storage out of the cage space. This would increase the cage space for the rats and also minimize obstruction to airflow.

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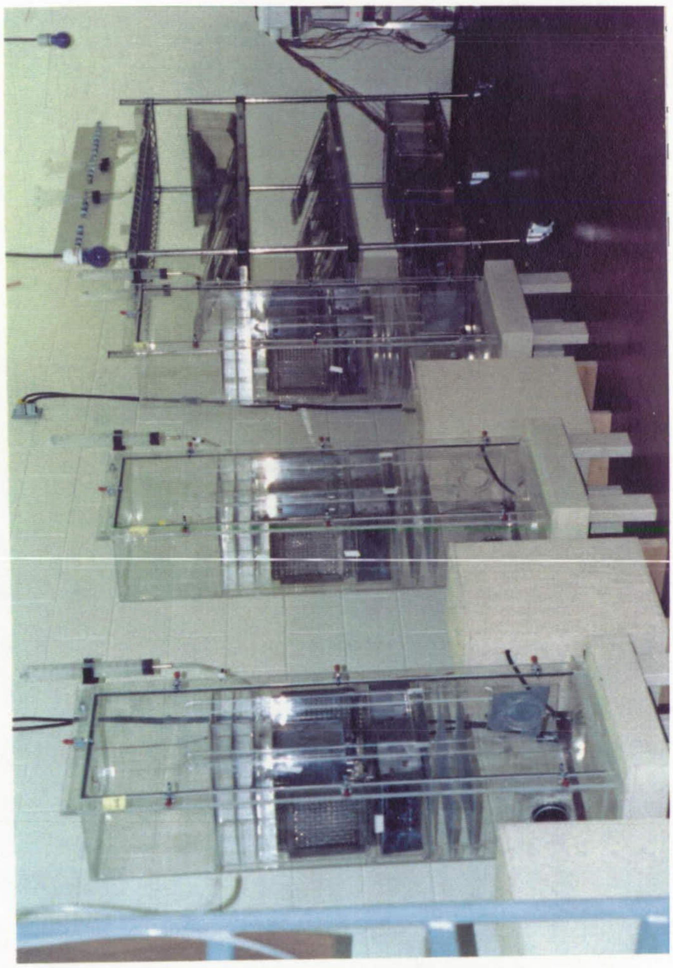
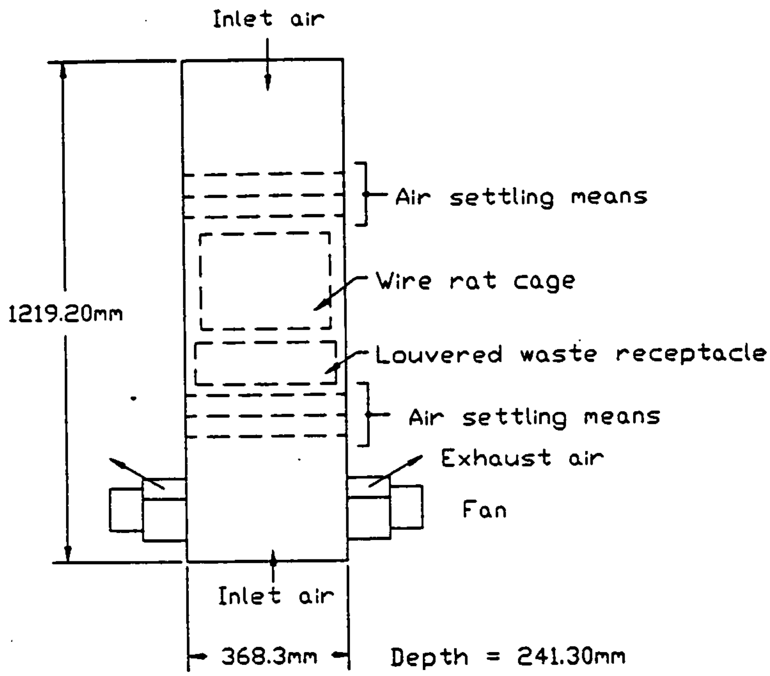
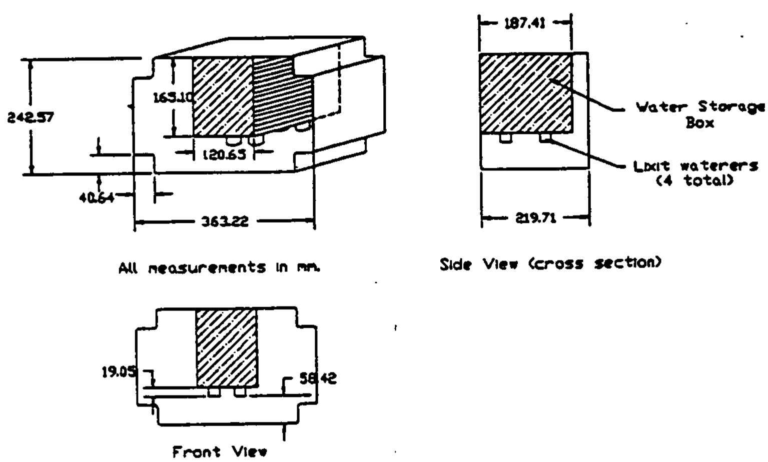


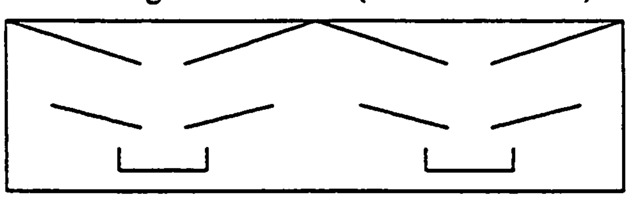
Figure 1. Mock animal enclosure modules (MAEM).



(a) Mock Animal Enclosure Module (MAEM).



(b) MAEM Cage Dimensions (all units are mm)



(c) MAEM Louvered Waste Receptacle

Figure 2. Schematic diagrams of (a) mock animal enclosure module (MAEM), (b) MAEM cage, and (c) MAEM louvered waste receptacle.

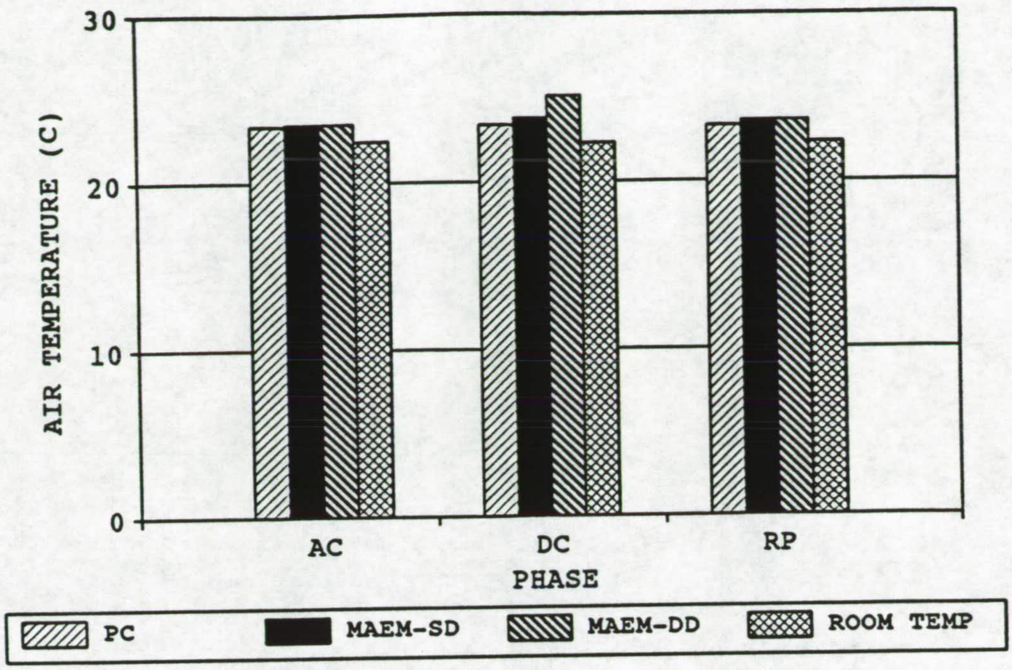


Figure 3. Overall mean cage air temperatures.
SEMs are given in Tables 1 and 3 of report by Maghirang and Riskowski.

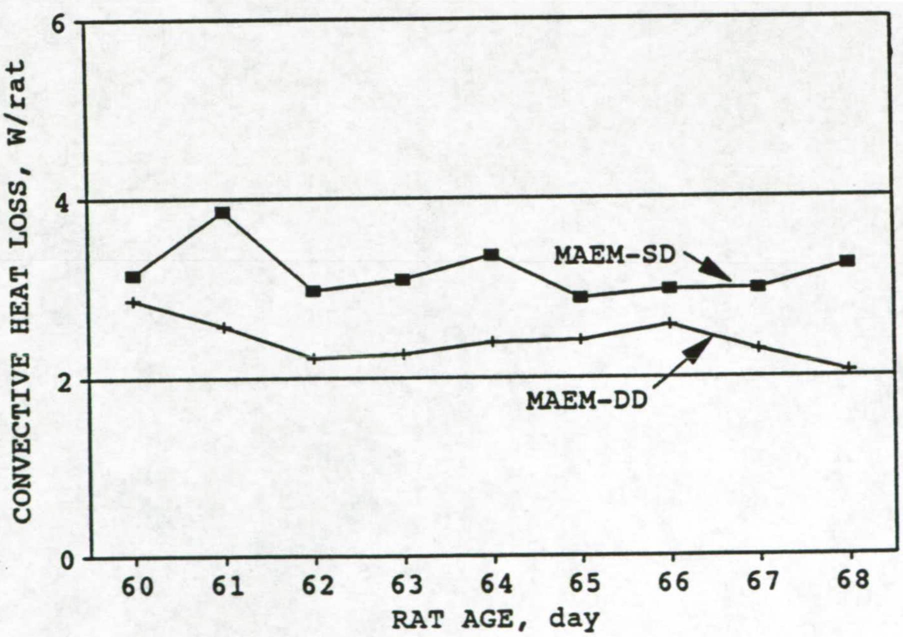


Figure 4. Estimated heat loss from rats in MAEMs.

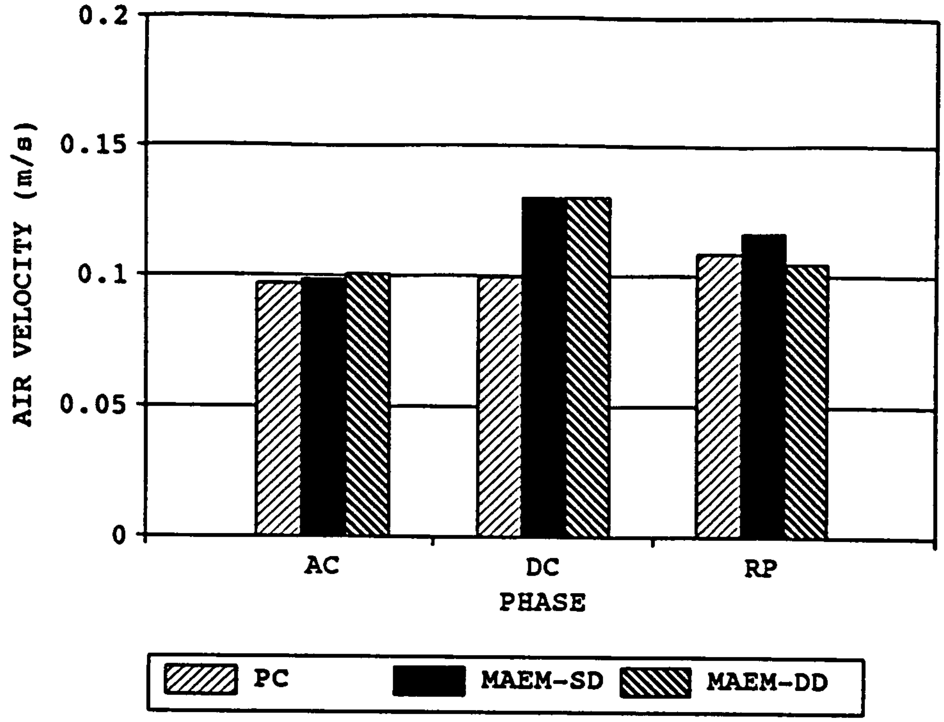


Figure 5. Overall mean air velocities approaching the cages.
SEMs are given in Table 4 of report by Maghirang and Riskowski.

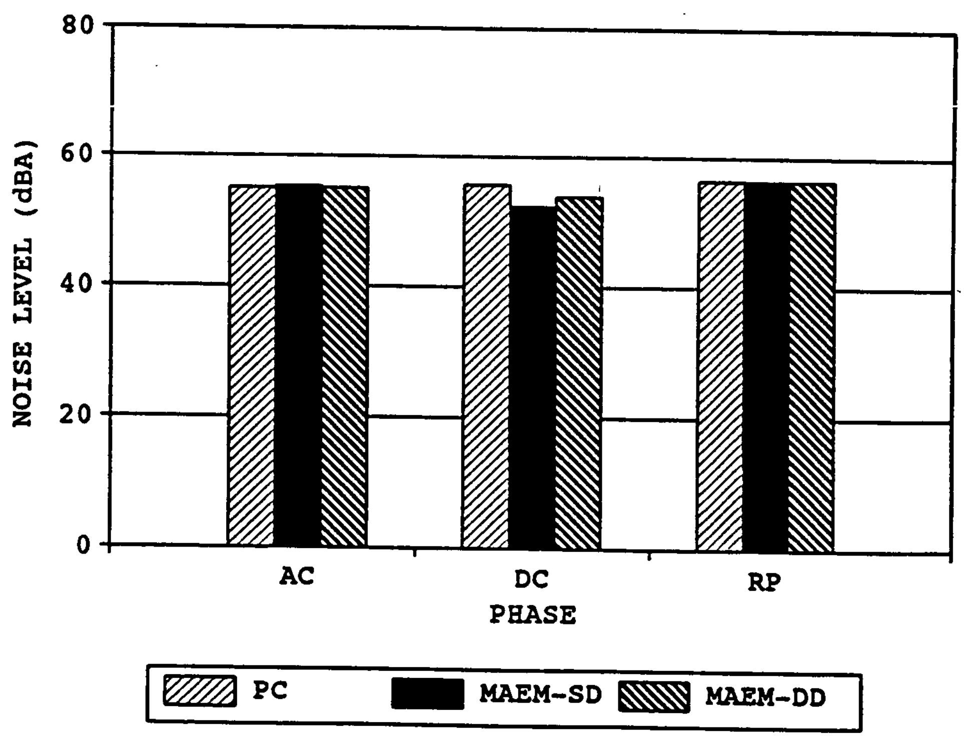


Figure 6. Overall mean noise levels within the cages.
SEMs are given in Table 5 of report by Maghirang and Riskowski.

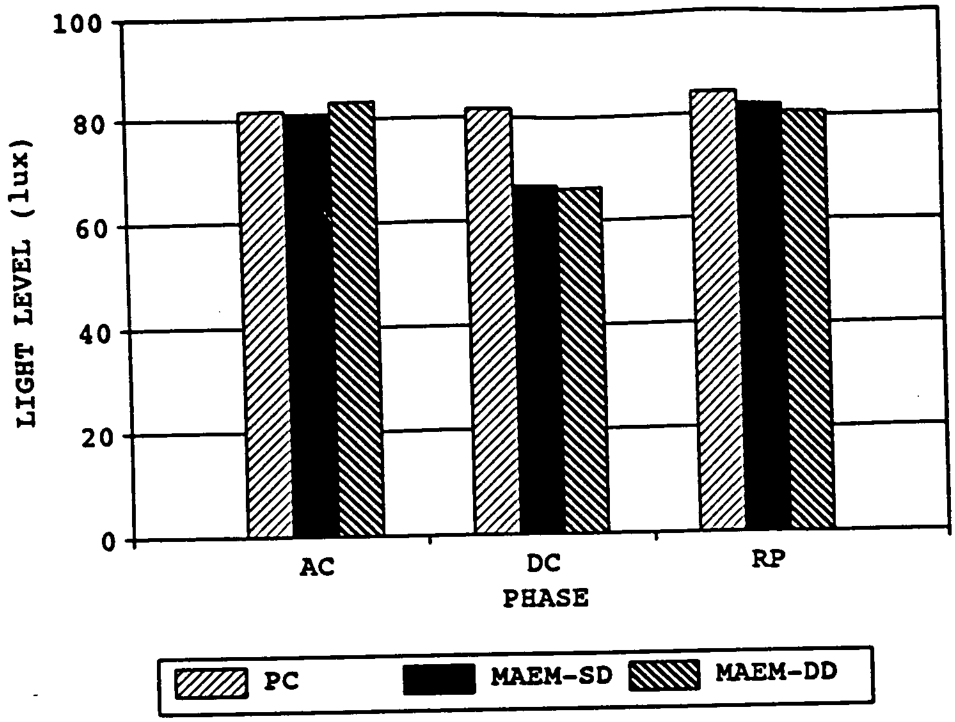


Figure 7. Mean light levels within the cages during light hours.
SEMs are given in Table 6 of report by Maghirang and Riskowski.

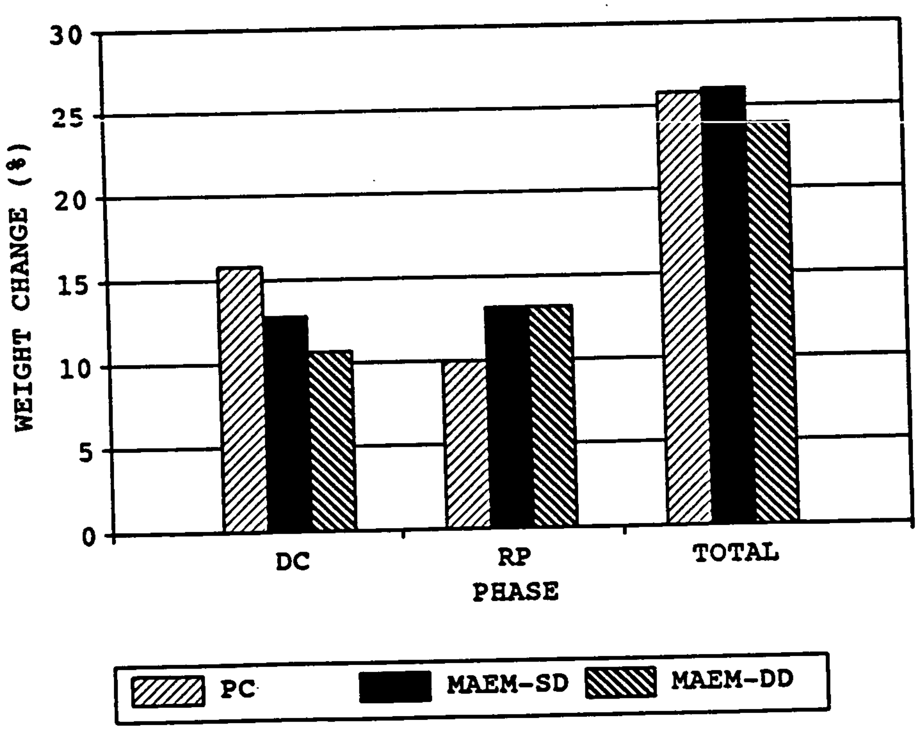


Figure 8. Percent body weight change for DC and RP phases.
Cage means are reported. SEMs are given in Tables 2 and 3 of report by Sebek.

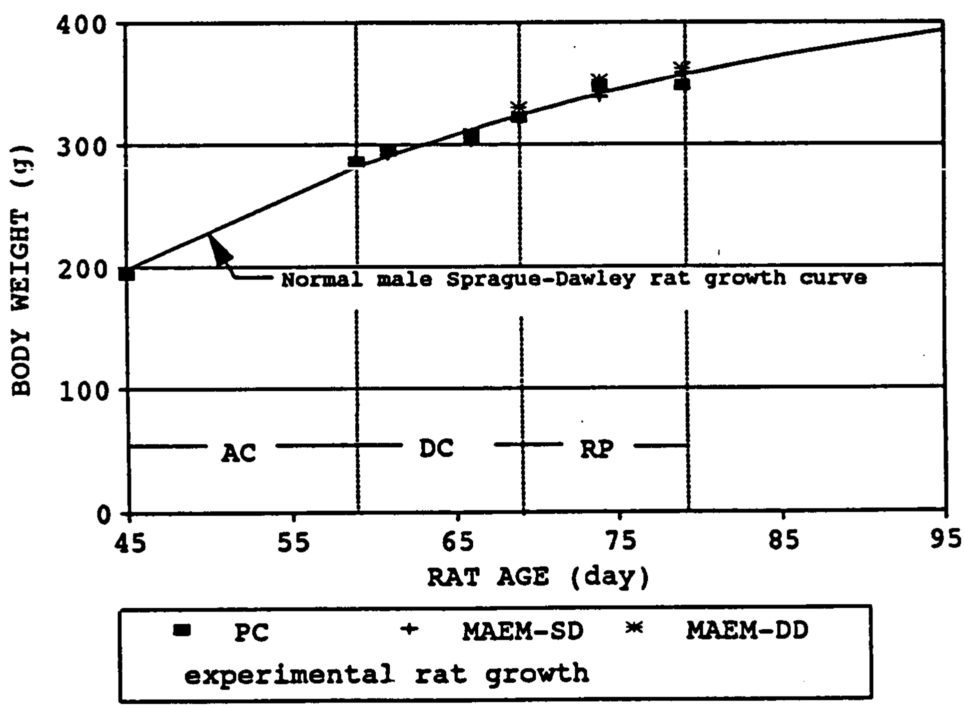


Figure 9. Normal growth pattern of male Sprague-Dawley rats relative to experimental findings.
Source: Harlan Sprague-Dawley, Inc.

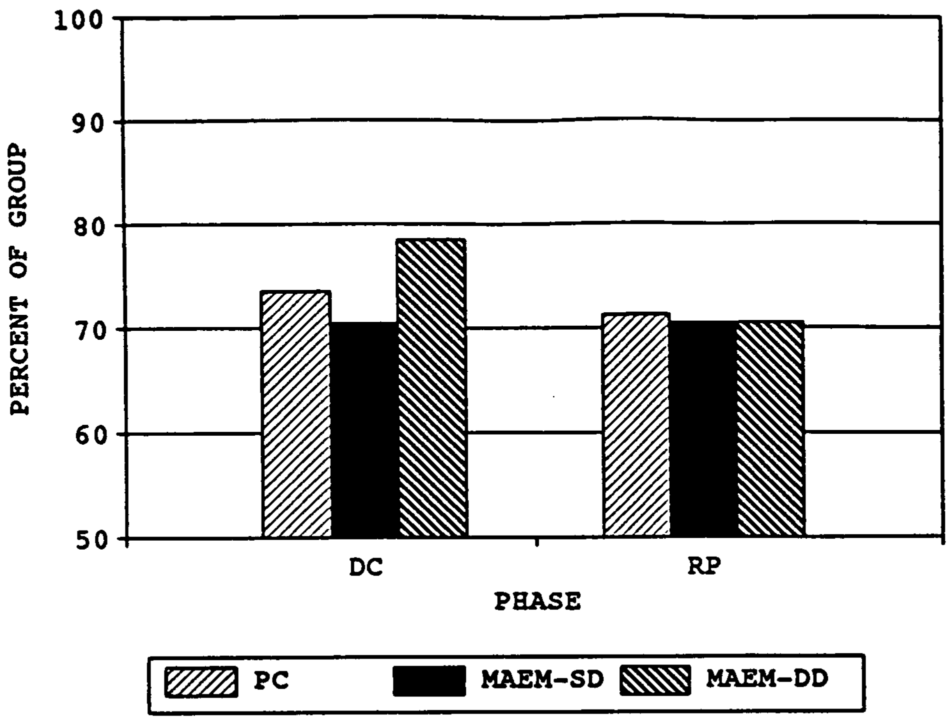


Figure 10. Lying activity by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

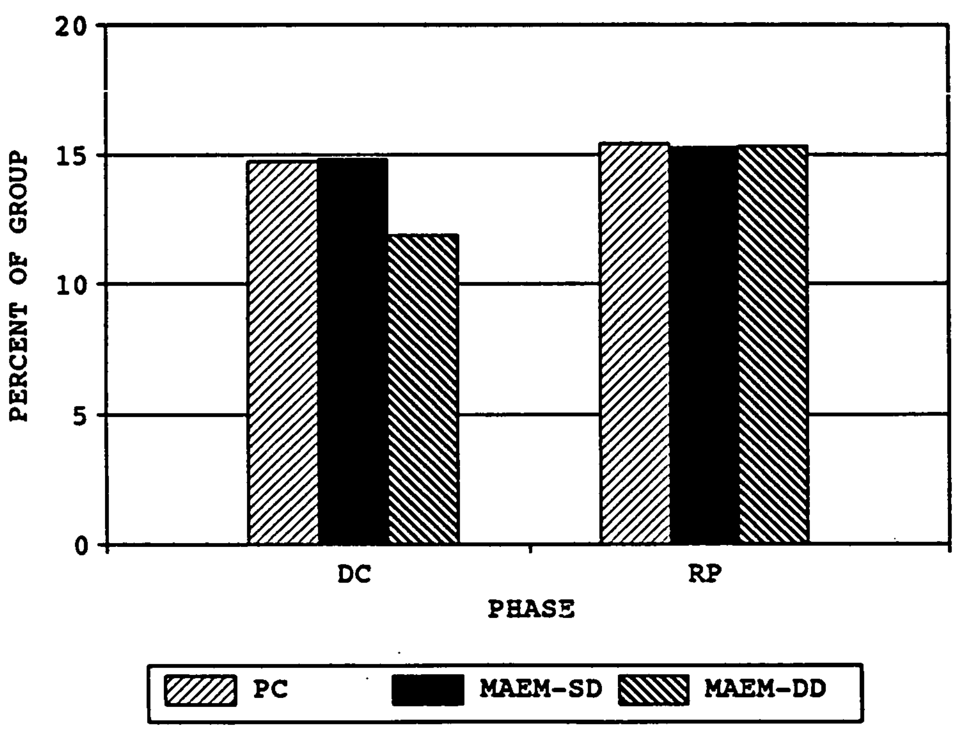


Figure 11. Sitting activity by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

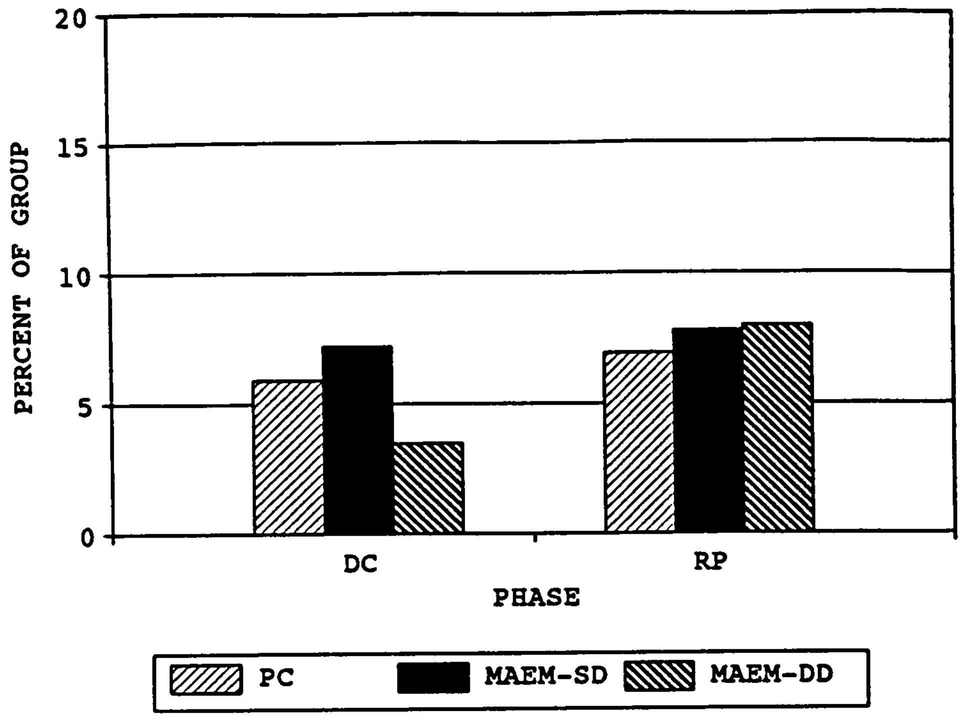


Figure 12. Normal standing activity by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

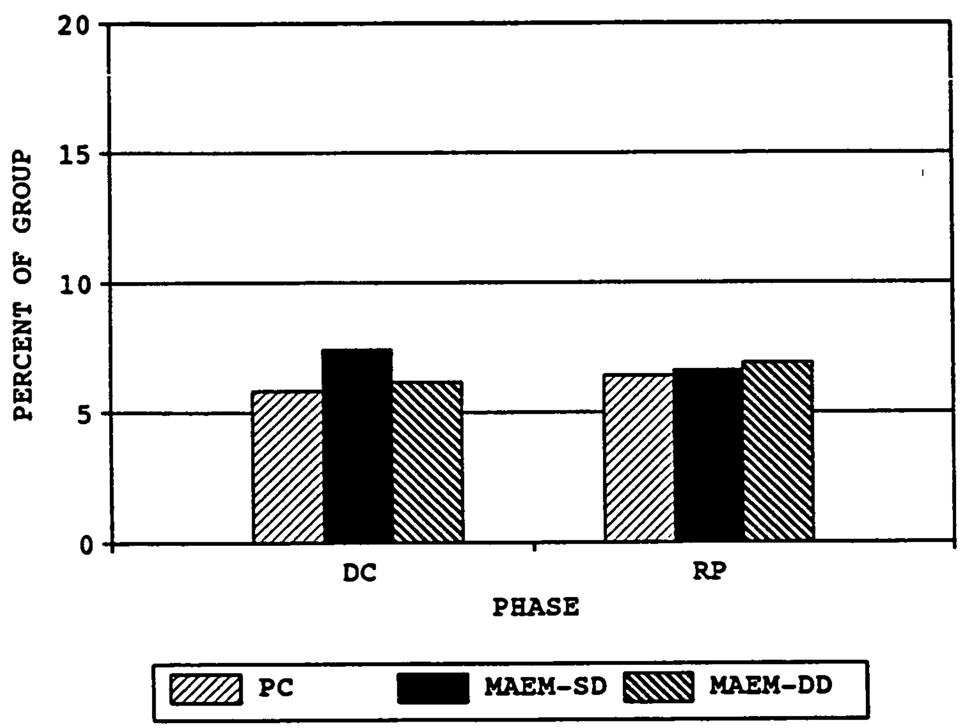


Figure 13. Erect standing activity by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

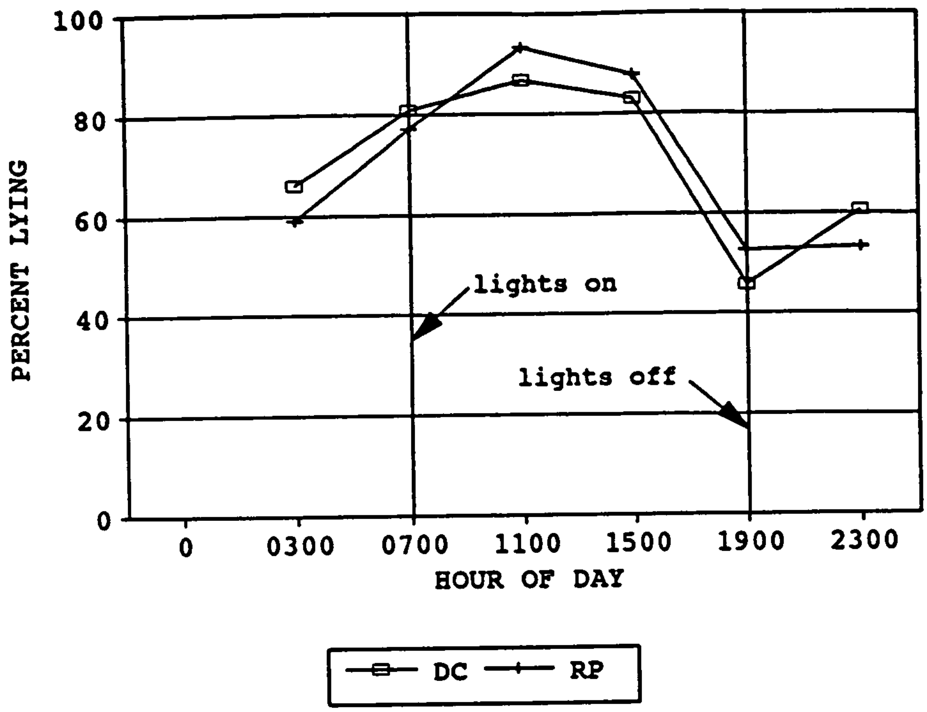


Figure 14. Diurnal lying activity for the PC treatment.

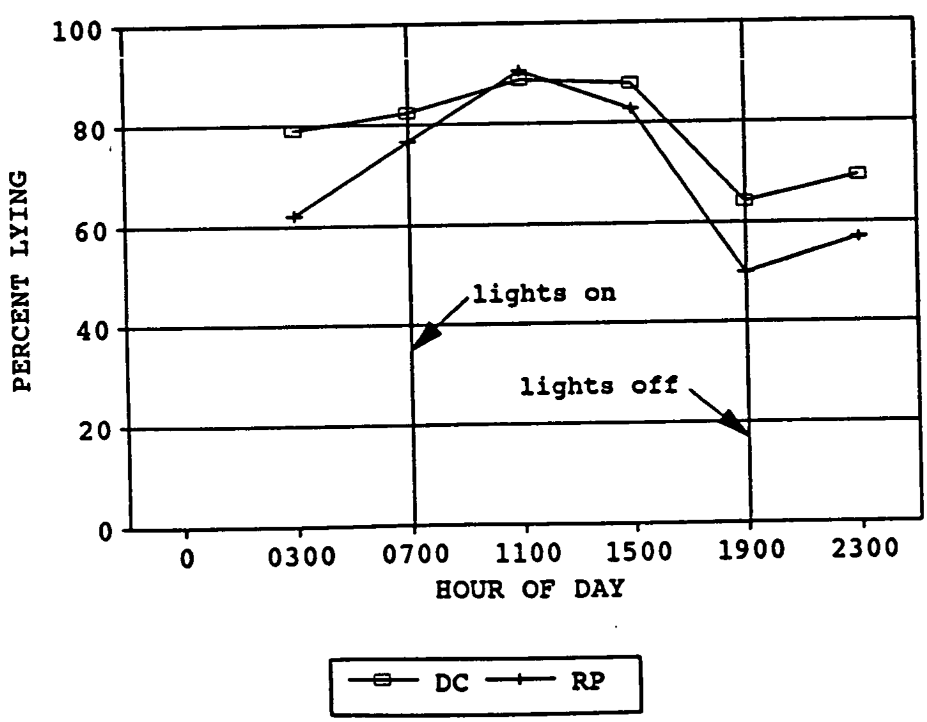


Figure 15. Diurnal lying activity for the MAEM-DD treatment.

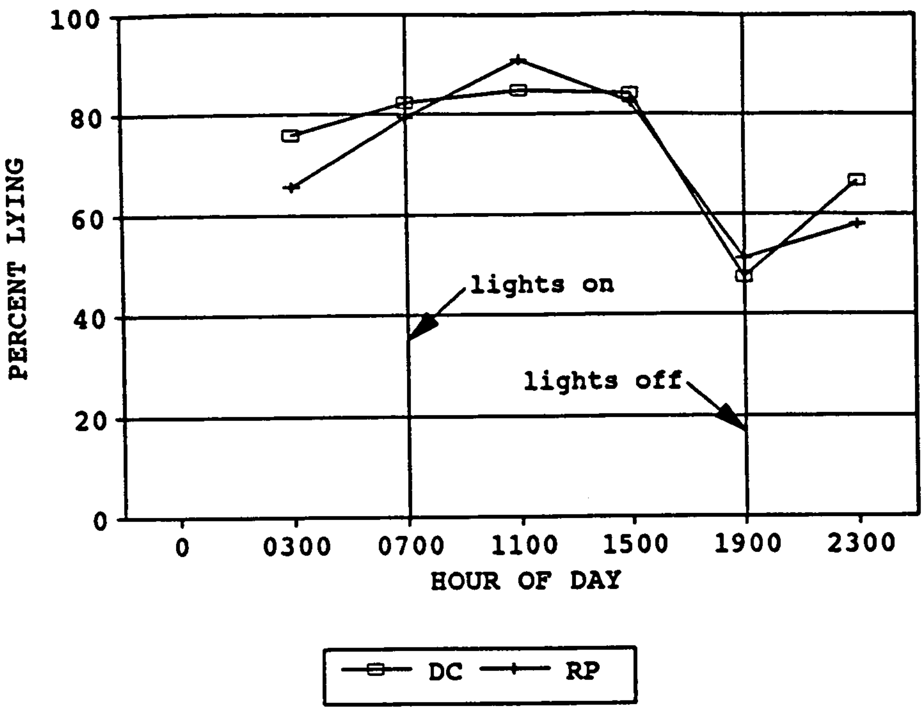


Figure 16. Diurnal lying activity for the MAEM-SD treatment.

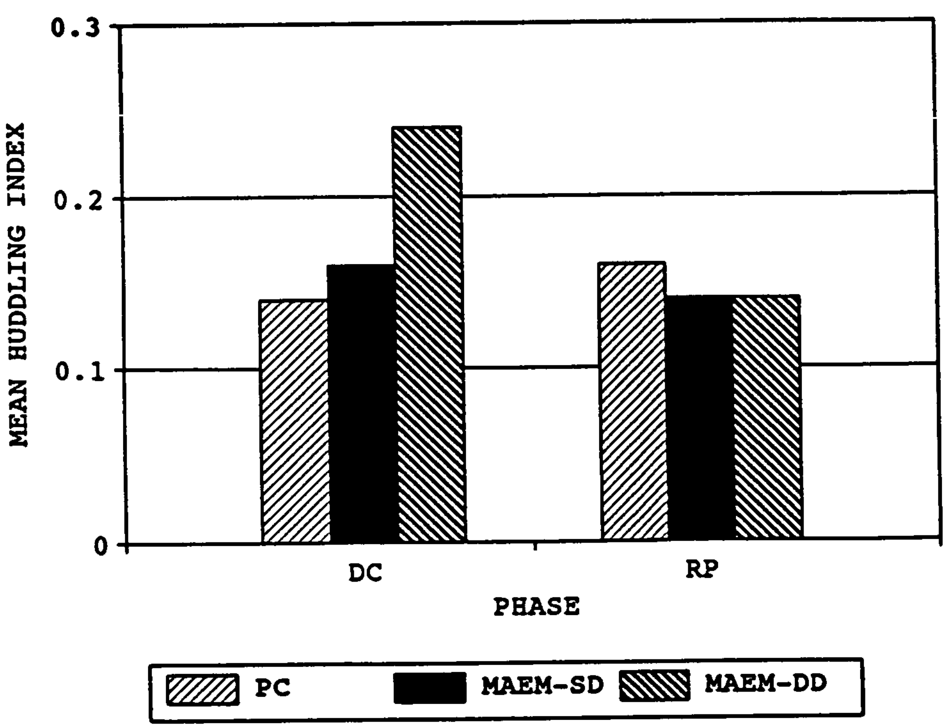


Figure 17. Mean huddling index by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou

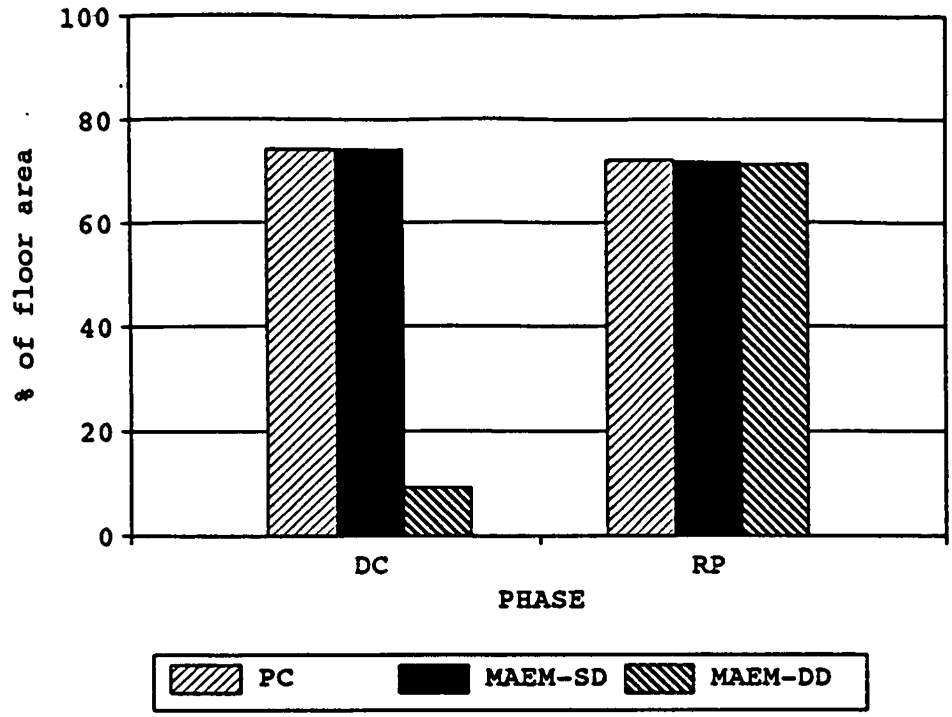


Figure 18. Free floor area by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

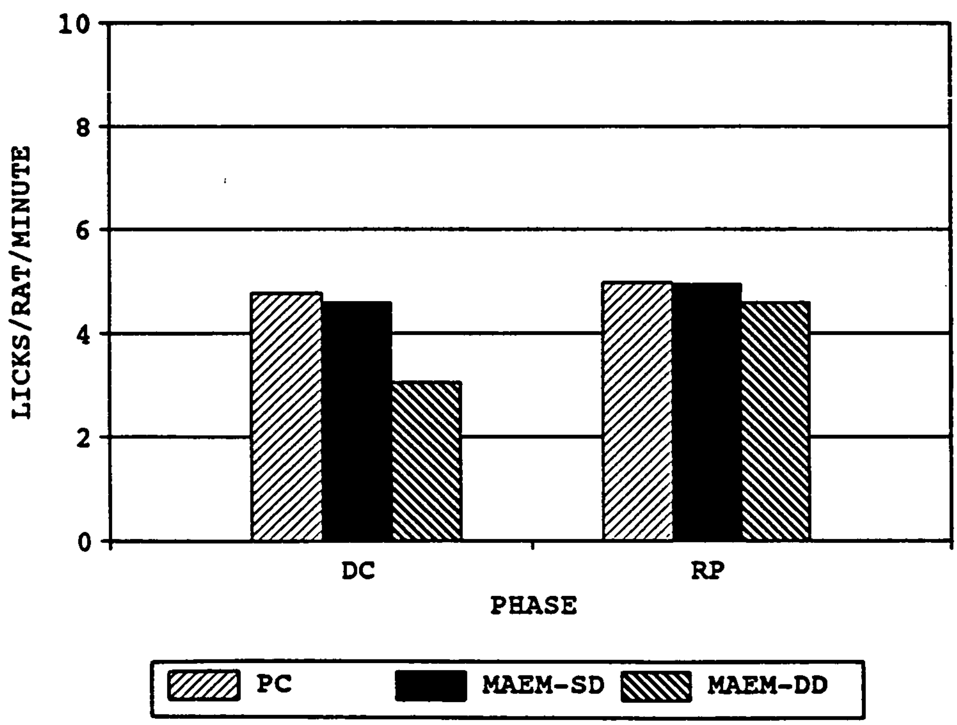


Figure 19. Licking frequency by cage type and phase.
SEMs are given in Tables 1 and 2 of report by Stricklin and Gonyou.

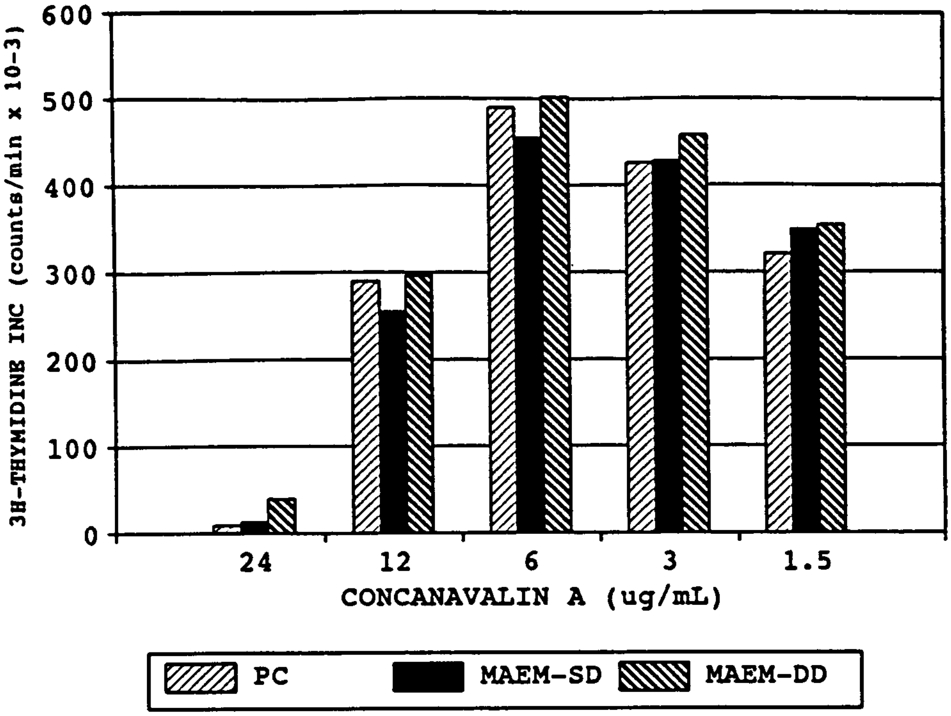


Figure 20a. Lectin-induced lymphocyte proliferation, by treatment for DC. SEMs are given in Table 1 of report by McKee.

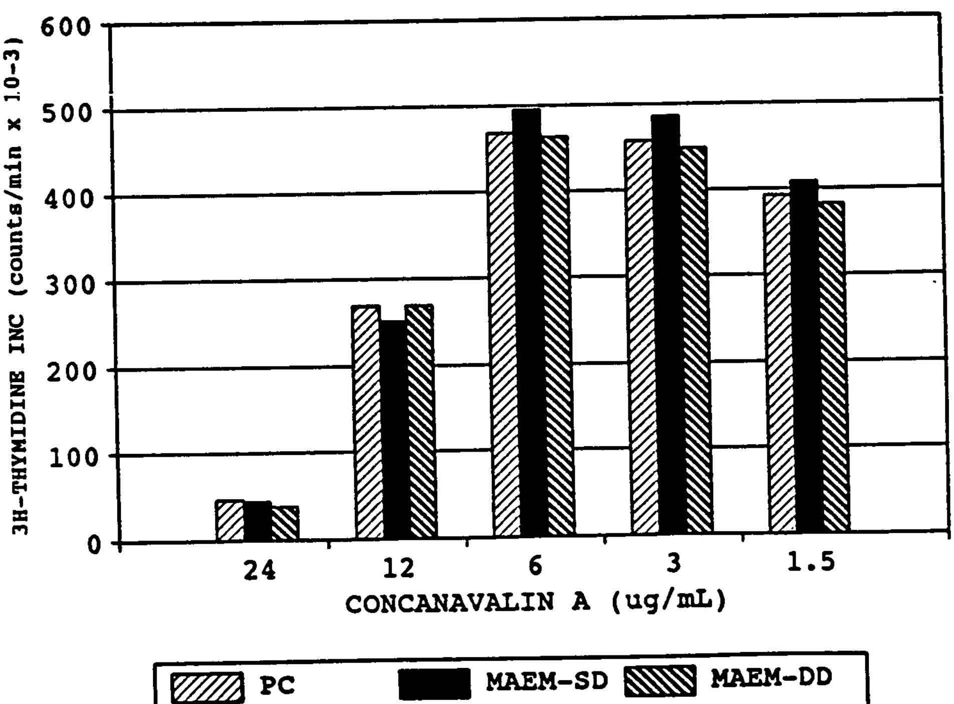


Figure 20b. Lectin-induced lymphocyte proliferation, by treatment for RP. SEMs are given in Table 1 of report by McKee.

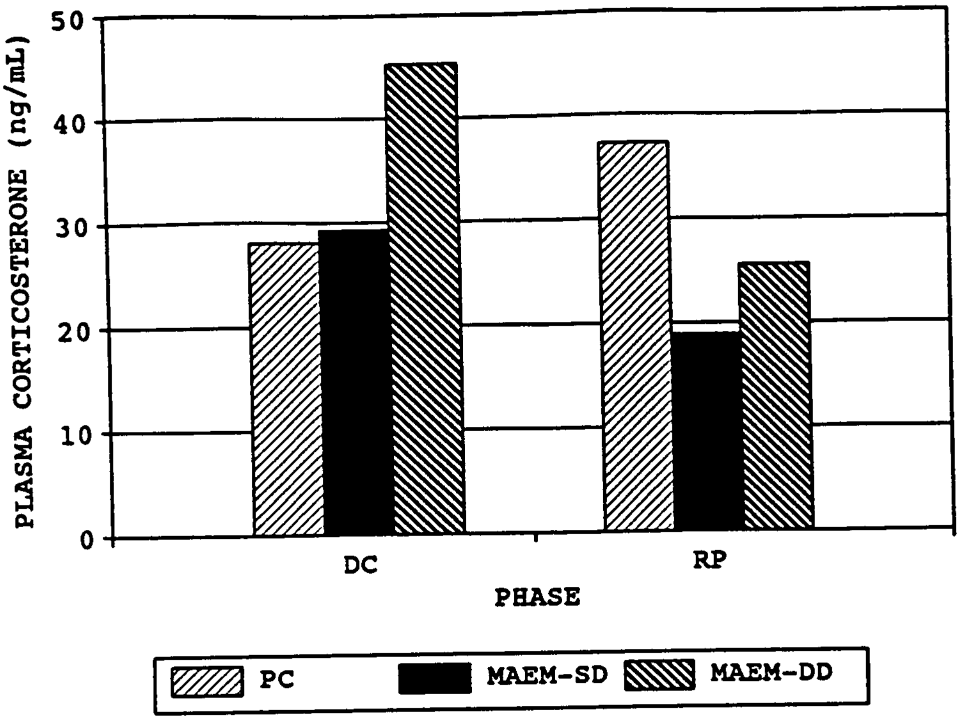


Figure 21. Plasma corticosterone, by treatment, for DC and RP.
SEMs are given in Table 2 of report by McKee.

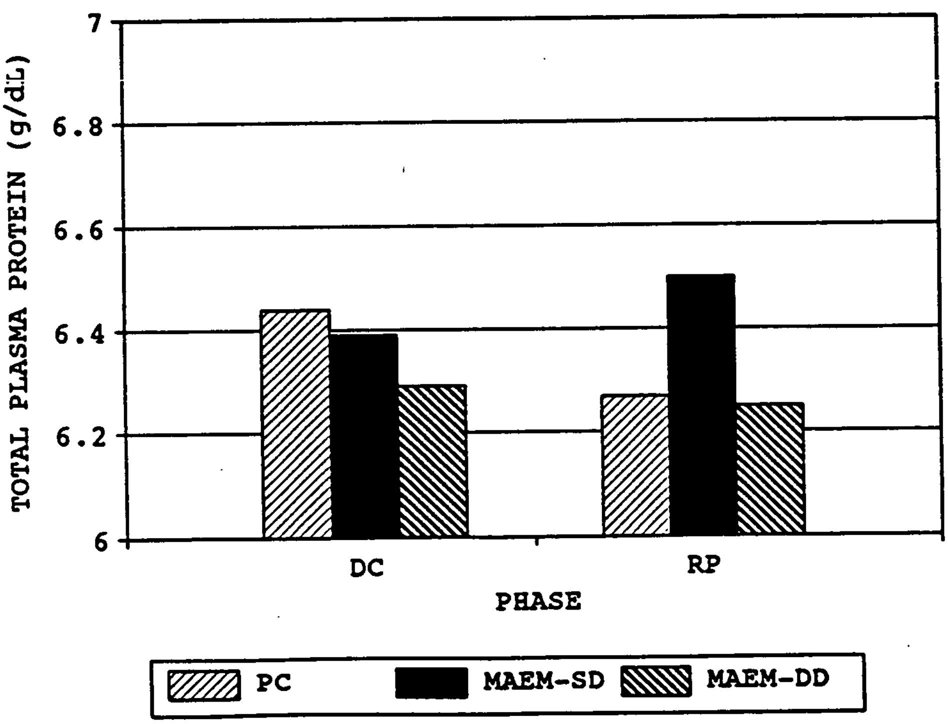


Figure 22. Total plasma protein, by treatment, for DC and RP.
SEMs are given in Table 3 of report by McKee.

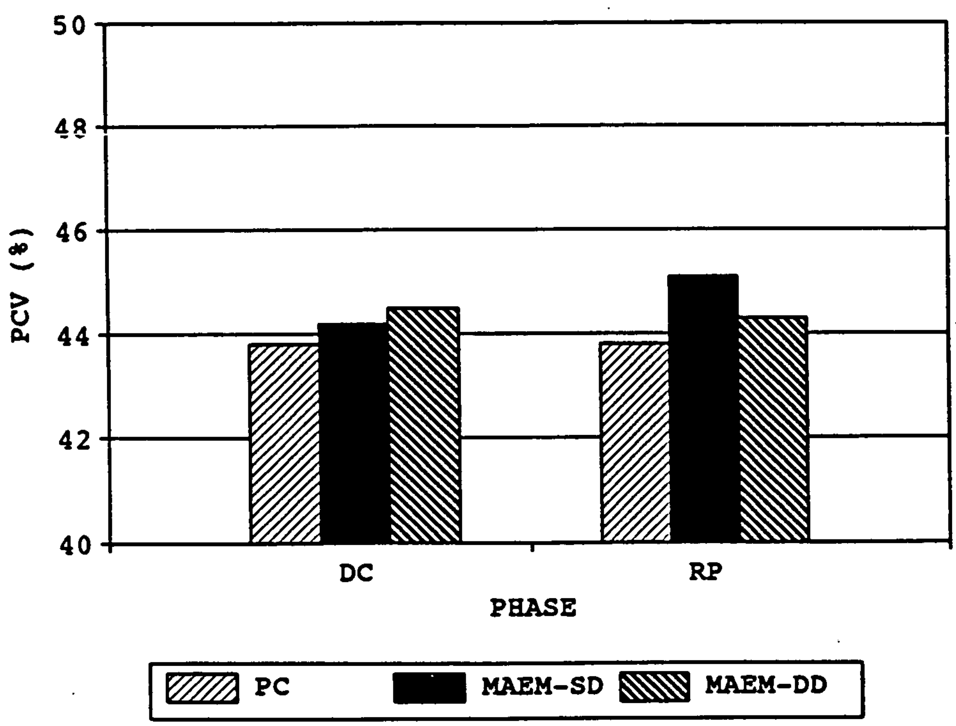


Figure 23. Percent cell volume, by treatment, for DC and RP.
SEMs are given in Table 5 of report by McKee.

THE EFFECT OF DOUBLE DENSITY CAGING DURING SPACE SHUTTLE TRANSPORT OF LABORATORY RATS: ENVIRONMENT

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Introduction

Numerous factors may influence the behavior and physiologic responses of laboratory animals. Regardless of the species of animals, their behavior, physiology, and affectivity can be influenced by physical (e.g., heat, humidity, sound, light, air contaminants), organismic (e.g., sex, age, size, genetics), and adaptive (e.g., activity, body covering, social) factors (Rohles, 1971). During space shuttle launch and transport, both cage type and cage population density could influence the behavior and physiologic responses of rats. The influence of density (4 vs. 8 rats/cage) in the National Aeronautics and Space Administration (NASA) Animal Enclosure Modules (AEM) on behavior and physiologic responses of the rats was investigated. The standard polycarbonate cage and the Mockup of the Animal Enclosure Module (MAEM) were compared.

An important aspect of this study is the rat macroenvironment (room) and microenvironment (cage). Environmental conditions within laboratory animal macroenvironments (rooms) and microenvironments (cages) are important not only for human operator and animal well-being and but also for reliability of experimental procedures (Besch, 1980). Thus, environmental parameters were controlled in this study to ensure uniform thermal environment among cages throughout the experiment and that unintended stressors were not introduced. A comparison of the environmental conditions among treatments is presented. Sensible heat production of laboratory rats in the MAEMs was estimated and compared with published values.

Materials and Methods

The study consisted of three phases: (1) acclimation (AC), (2) density challenge (DC), and (3) recovery (RP). During the AC and RP phases, rats were housed in groups of four in standard polycarbonate cages (shoe box with mesh cover) at recommended density (NIH, 1985). During the DC phase, the rats were assigned randomly to three treatments: (1) PC: 4 rats/polycarbonate cage, (2) MAEM-SD: 4 rats/mock animal enclosure module, and (3) MAEM-DD: 8 rats/mock animal enclosure module. There were five replications of each treatment and all treatments were represented during each replication.

All cages (polycarbonate and mock animal enclosure modules) were housed in the same laboratory animal room during all three phases. The room was a standard laboratory animal room located in the Plant and Animal Biotechnology Laboratory, University of Illinois. The room had air inlets on the ceiling and an exhaust outlet on one of the walls (Fig. 1a). The polycarbonate cages and the MAEMs were arranged as shown in Figure 1b.

The ventilation rate and thermal environmental conditions in the laboratory room were controlled throughout the experiment. The air temperature and humidity in the room were controlled to desired levels (22°C and 50%, respectively). The light period was 12 h light and 12 h dark; the lights were turned on at 0700 h and turned off at 1900 h.

Caging system design and operation

Each polycarbonate cage had an area of 0.088 m^2 and a height of 0.20 m. The polycarbonate cages were assigned randomly to the two cage racks. Each rack had three shelves; rack 1 was used for 12 PC cages, whereas rack 2 was used for nine PC cages. Polycarbonate cages for replications 1, 2, and 3 were located in the top, middle, and bottom shelves, respectively. Cages for replications 4 and 5 were located in the same locations as those for replications 1 and 2, respectively.

Three MAEMs were constructed to simulate the AEM used by NASA for transport of rats in the space shuttle (Fig. 2a). The MAEMs were placed in the vertical position as they would in the middeck locker of a space shuttle. They were constructed and instrumented to develop a uniform air velocity of 0.13 m/s approaching the rat cage. Each MAEM had overall dimensions of 1.22 m x 0.28 m x 0.25 m (Fig. 2a). The outer housing was made of 6.4-mm-thick plexiglass.

The MAEM consisted of a rat cage, air settling means, and fans. Rats were housed in a stainless steel wire mesh cage located between two sets of air settling means. The cage had the same dimensions as the cage in the actual AEMs. Each air settling means consists of three sets of screens with different amounts of open area. The air settling means provided a uniform distribution of air velocity approaching the cage and reduced air turbulence. Rat wastes were collected on a series of louvered stainless steel sheets (Fig. 2b). The louvered sheets diverted liquid wastes to two lower pans while allowing air to flow between the sheets to the fans below. Wastes were removed from the collection louver pans after the DC phase. In contrast, the polycarbonate cage and its bedding were replaced every five days.

Two shaded pole blowers (Model 4C446, Dayton), each with a capacity of 70 L/s in free air, were used. These fans created a slight negative pressure inside the MAEM, thereby drawing air from the outside into the MAEM. Air moved unidirectionally from top to bottom of the rat cage and was exhausted through the fans. Initially, one blower was mounted on each of the two side walls. With this set-up, the noise level within the MAEM was about 75 dBA, which was higher than the noise level within the room. To reduce the noise level within the MAEM, the blowers were placed inside a separate fan box lined with acoustical foam. The foam served to absorb the noise and minimize vibration of blowers. There are other methods to reduce the noise level within the MAEM; for example, "quieter" fans may be used instead of the shaded pole blowers used in this study.

A fan motor speed controller (Model 57, Broan) was used to control ventilation rate and air velocity approaching the rat cage in the MAEM. Ventilation rate and velocity were also controlled using a bypass valve located at the bottom of the MAEM. A uniform air velocity of 0.13 m/s was achieved with two sets of air settling means. Three layers of perforated stainless steel sheets with 63%, 40%, and 33% openings formed a set of flow settling means at the top and bottom of the cage. The design of the fan outlet and flow settling means complied with the ASHRAE Standard for fan testing (ASHRAE, 1985).

Measurement and instrumentation

The following environmental parameters were monitored:

(1) Room air temperature (every 15 min)
(2) Air temperature within each cage (every 15 min)
(3) Room air relative humidity (twice daily, 0800 and 2000 h)
(4) Air velocity magnitude and direction approaching the top of each cage (twice daily, 0800 and 2000 h)
(5) Light intensity at the top center of each polycarbonate cage and at the sides of the MAEMs (twice daily, 0800 and 2000 h)
(6) Noise level within each cage (twice daily, 0800 and 2000 h)
(7) Ammonia level at the center of the room (once daily, 0800 h)
(8) Ammonia level within each cage (5th and 10th days, 1200 h, for both the DC and RP phases) before the PCs were replaced with fresh litter PCs
(9) Static pressure across the rats in the MAEMs (twice daily, 0800 and 2000 h).

All temperatures were measured using type T (copper-constantan) thermocouples connected to a data logger (Model 21X, Campbell Scientific Inc.). The data logger was interfaced to an IBM PC. Relative humidity was measured with an aspirated psychrometer (Cole Parmer). Room air temperature and relative humidity were recorded continuously with a microprocessor-based temperature/relative humidity recorder (Model CT485RS, White Box), which had a ±1°C and ±3% accuracy for temperature and relative humidity, respectively. Air velocity was measured with an omnidirectional probe (Model 8470, TSI Inc.), which was accurate for the relatively low air velocities encountered in laboratory animal cages (accuracy: ±6.5% of the reading for velocities less than 0.18 m/s). The probe was connected to a display/power supply unit (Model 8910, TSI Inc.). Light intensity levels were measured with a light meter (Model P401025, Extech) and noise levels with a digital sound level meter (Model 2700, Quest). These instruments were accurate to within ±5% of the reading and ±1 dBA, respectively. Ammonia level was determined with amine gas detector tubes (MSA) and a calibrated air volume pump. These detector tubes had a minimum level of detectability of 1 ppm. Static pressure drop across the rats was measured with a micromanometer, which had a 0.001 in water column sensitivity.

Statistical analysis

The analysis of variance (ANOVA) procedure (SAS, 1985) was used to investigate the differences on environmental parameters among the three treatments. Separate analyses were performed for the three phases. The mean values of each parameter for each treatment and replication were used in the analysis (number of observations=15). For air velocity, noise level, and light intensity level, the average of the 0800 h and the 2000 h means were used. The model included Treatment (degrees of freedom=2), Replication (4), and Replication x Treatment (8); Replication x Treatment served as the error term. A level of significance of 5% was used to test for differences among the treatments.

Results and Discussion

Ventilation, temperature, and relative humidity in the room

The room air exchange rate was determined with a flowhood connected to a multimeter (Shortridge Instruments). The rate was 12 air changes per hour (ach), which satisfied the ventilation requirements of 10 to 15 ach and 0.385 L/s per rat (ILAR, 1977).

Mean air temperature at the center of the room was practically constant for all phases and replications (Table 1). Overall mean air temperature at the center of the room varied from 21.8 to 22.8°C. Overall mean air temperatures for the five replications are shown in Figure 3. The overall mean air temperature for the entire experiment was 22.2 (±0.1)°C.

The mean humidity among the three phases for each replication and among the five replications for each phase did not differ (Table 2). Overall mean relative humidity for the five replications ranged from 48 to 60% (Fig. 3). The overall mean relative humidity for the entire experiment was 54 (±1) %.

Ammonia concentrations in room and in cages

The ammonia level at the center of the room for all days was not detectable (well below 1 ppm) with the detector tubes. The low ammonia level indicated that the ventilation and cleaning frequency were sufficient to maintain a desirable condition within the room. A five-day cleaning cycle was used throughout the experiment, i.e., the polycarbonate cage with litter was replaced every 5 days.

During the DC phase, ammonia level in PC cages was detected in one cage (2 ppm) during the 10th day for replication 4 and in two cages (2 and 30 ppm) during the 5th day for replication 5. Ammonia levels in MAEMs with single density were negligible for all replications. For MAEMs with double density, an ammonia level of 2 ppm was observed during the 5th day of the DC phase of replications 1 and 3, and during the 10th day for replication 4. During the RP phase, where rats were housed in polycarbonate cages, an ammonia level of 7 ppm was observed in one cage of replication 5 during the 5th day; the ammonia level (less than 1 ppm) was not detectable with the detector tubes in all other cages.

Cage air temperatures

Mean cage air temperatures by treatment, phase, and replication are summarized in Table 3. For each replication, mean temperature in PC cages during the AC and RP phases were not considerably different among the three treatments. The PC cages had a mean temperature variation of less than 1.3 °C. During both the AC and RP phases, where all rats were housed in polycarbonate cages, mean cage air temperatures did not significantly ($P > 0.05$) differ among the three treatments (Fig. 4).

During the DC phase, small differences in mean temperature were observed among the treatments for all replications. MAEMs with double density had the highest mean temperature; whereas, PC cages had the lowest mean temperature. Ranges of mean temperature during the DC phase were 23.3 to 23.5, 23.1 to 24.6, and 24.7 to 25.7 °C for the PC, MAEM-SD, and MAEM-DD treatments, respectively. Mean cage air temperature for the MAEM-DD treatment was significantly ($P < 0.05$) higher than that for either the MAEM-SD or PC treatments. No significant ($P > 0.05$) difference was observed between the mean temperatures for MAEM-SD and PC treatments. The higher temperature for the MAEM-DD treatment can be accounted for by the higher heat generated in MAEM-DD than either in the MAEM-SD or in the PC treatments. The MAEM-DD had eight rats; whereas, the MAEM-SD and the PC had four rats each. The difference among the treatments was statistically significant; however, it was not physiologically important and would not influence the rats.

Cage air temperatures were slightly higher than room air temperature (Table 1; Fig. 4) because of heat production by the rats. For all PC cages, mean daily cage air temperatures were 0.1 - 2.5 °C higher than the air temperature at room center. For the MAEMs with single density, mean daily cage air temperature was 0.3-2.8 °C higher than the mean room air temperature for all replications. For the MAEMs with double density, mean daily cage air temperature was 0.3-4.7 °C higher than room air temperature.

Air velocity approaching the top of cage

Air velocity approaching the cage rather than within the cage was considered. The air velocity within polycarbonate cages was lower than the approach air velocity; a smoke test showed that the smoke hardly moved within the cage, indicating that there was negligible air movement within the cage. Zhang et al. (1992) observed negligible air velocity (less than 0.05 m/s) within polycarbonate cages. In the MAEMs, the approach air velocity was close to the air velocity within the cage.

The PC cages exhibited a higher variability in air velocity than the MAEMs (Table 4). For the PC cages, mean velocity varied from 0.07 to 0.18 m/s. For the MAEMs, mean velocity ranged from 0.12 to 0.14 m/s fpm. The air velocity at the top center of the PC cages depended largely on room air movement, which, in turn, depended on numerous factors such as room ventilation rate, cage rack location, obstructions to flow, temperature, etc. On the other hand, the air velocity in the MAEMs was fairly constant and independent of room air movement because the MAEMs were ventilated individually.

Overall mean air velocities for the three treatments are shown in Figure 5. Like cage air temperature, during both the AC and RP phases, mean air velocities at the top of the polycarbonate cages for the three treatments did not significantly ($P > 0.05$) differ. However, during the DC phase, the overall mean air velocity for either the MAEM-SD (0.13 m/s) and MAEM-DD (0.13 m/s) were significantly ($P < 0.05$) higher than that for the PC treatment (0.10 m/s). As noted above, air velocities in the MAEMs were controlled; whereas, air velocities approaching the polycarbonate cages were not and depended on room air movement. The observed difference in air velocity can have an influence on the behavior and physiogic response of rats but was an inherent part of the treatments; the influence of air velocities on laboratory rat responses deserves more detailed study.

Noise levels

Noise levels within the cages (PCs and MAEMs) were much lower than the maximum allowable noise level of 85 dBA in animal facilities. Mean noise levels within the cages varied from 50 to 65 dBA (Table 5). Overall mean noise levels for the three treatments were uniform and ranged from 52 to 57 dBA (Fig. 6). There were no significant ($P > 0.05$) differences in mean noise levels among the three treatments for all three phases.

Light intensity levels

The light intensity at the top center of each PC cage and at the sides of the MAEMs was measured with a light meter. For the PC cages, ranges of mean light intensity levels at 0800 h were 45 to 68, 32 to 42, 30 to 47, 42 to 59, and 35 to 43 lux for replications 1, 2, 3, 4, and 5, respectively (Table 6). Differences among replications can be accounted for by cage location in the cage rack. The PC cages for replications 1 and 4, 2 and 5, and 3 were located at the top, middle, and bottom shelf of a three-shelf rack, respectively. During the dark period, only blue light bulbs were used for videotaping cages; mean light levels ranged from 4 to 11 lux. The mean light levels at the sides of the MAEMs varied from 200 to 251 during the lighted period and from 3 to 4 during the dark period.

Light levels within the cages (along the floor) were measured at five locations in the PCs and at six locations in the MAEMs. Mean light level inside the PC was higher than light level at the top of the PC because light can be transmitted through the sides of the PC. Within the cage of MAEMs, light level was considerably lower than the the light level at the sides of MAEMs because of low light transmission to the cage due to the presence of food bar plates lining the cage sides and the air settling means on top of the cage. Mean light intensity levels inside the cages during the lighted period ranged from 67 to 87 lux. During the AC and RP phases, no significant ($P > 0.05$) difference in mean light levels existed among the treatments. During the DC phase, mean light levels inside the MAEMs were slightly lower than mean light levels inside the PCs.

Static pressure

Static pressure accross the rats in the MAEMs was measured at 0800 and 2000 h using a micromanometer. Static pressure drop accross the rats for all MAEMs was negligible.

Sensible heat production of laboratory rats

Laboratory rats produce various quantities of metabolic heat, depending on body weight, amount and type of feed consumed, and environmental conditions. A portion of the heat is dissipated in the form of sensible heat and the rest as latent heat. Sensible and latent heat production can be estimated from an energy and moisture balance. Only sensible heat was considered in this study; under normal conditions, sensible heat production is twice the latent heat production (Besch, 1973; Woods et al., 1972).

The sensible heat balance in the MAEM may be represented as

$$q - q_v - q_{cd} - q_r = 0 \quad (1)$$

where

q = sensible heat produced by the rats, W
q_v = sensible heat loss by convection, W
q_{cd} = sensible heat loss by conduction, W
q_r = sensible heat loss by radiation, W

Sensible heat transfer in the MAEM was purely convective so that sensible heat production of rats can be determined by estimating convective heat loss. Convective heat transfer can be represented by:

$$q_v = Q\rho C_p \Delta T/n \quad (2)$$

where

q_v = convective heat loss, W/rat
Q = ventilation rate in the MAEM, m^3/s
ρ = density, kg/m^3
C_p = specific heat, J/kg-°K
ΔT = difference between the incoming and the outgoing air temperature, °C

n = number of rats in the cage

Using equation 1, mean daily sensible convective heat transfer rates were determined for rats in MAEMs during the DC phase. Mean daily convective heat losses averaged across replications for MAEM-SD and MAEM-DD treatments are shown in Figure 8. For both treatments, convective heat losses did not vary significantly ($P > 0.05$) with rat age. Rats in the MAEM-DD treatment had lower convective heat loss than rats in the MAEM-SD treatment. Convective heat losses for MAEM-SD and MAEM-DD varied from 2.87 to 3.86 W/rat and from 2.05 to 2.87 W/rat, respectively; overall mean convective heat losses were 3.16 and 2.41 W/rat. The lower heat loss in the MAEM-DD treatment may be explained by the huddling behavior of rats; they tended to pile on top of one another reducing the convective surface area and consequently the convective heat transfer rate. It appears that heat load in the MAEM may be decreased by increasing the number of rats in the group.

Sensible heat generated by laboratory rats during normal activity can be approximated using the following formula (ASHRAE, 1991):

$$\text{Sensible heat production} = (0.67)\ 2.5\ M \qquad (3)$$

where

M = metabolic rate of the animal, W/rat = $3.5W^{0.75}$

W = mass of the animal, kg

Using equation 3, sensible heat generated by a 300-g rat would be 2.38 W. This value is close to the observed heat loss for rats in MAEM-DD rats, but is 33% lower than the observed value for MAEM-SD rats. Numerous factors may influence heat production and/or convective heat transfer rate of laboratory rats and differences in these factors may account for this discrepancy. For example, thermal environmental conditions (temperature, humidity, air velocity) would affect convective heat transfer rate. Rats in the present study were maintained in a vertical orientation relative to the air flow and this too may affect the rate of convective heat loss. Orientation would alter both the characteristic dimension of the animal and the wind penetration and turbulence effects on the hair coat. The influence of environmental and other factors (e.g., number of rats in the group) on heat production of laboratory rats needs further study.

Summary and Conclusion

A study was conducted to determine the influence of cage type (polycarbonate vs. mockup of the animal enclosure module) and cage population density (normal vs. double) in the MAEM on the cage microenvironment and on rat growth and physiologic responses. MAEMs were constructed and instrumented to simulate the AEM used by NASA for transport of rats in the space shuttle. These MAEMs had precisely controlled unidirectional (from top to bottom of cage) airflow. Environmental conditions (room air temperature and humidity, noise, and light level) were controlled throughout the experiment to ensure uniform conditions for all cages and replications.

Analysis of data showed that:

(1) There were no significant differences among treatments in mean cage air temperatures, air velocities, noise, and light levels during acclimation and recovery phases where all rats were housed in polycarbonate cages.

(2) During the density challenge phase, cage type (PC vs. MAEM) did not have any significant influence on cage air temperature and noise level. Ammonia level in the MAEM was negligible; whereas, the ammonia level in the PC ranged from negligible to 30 ppm.

(3) Rat density (4 vs. 8 rats/cage) in the MAEM did not have any significant effect on noise, light, and ammonia levels or on air velocity. The difference in mean cage air temperature for the MAEM with double density (25.1°C) and for the MAEM with single density (23.8°C) was significant ($P < 0.05$).

(4) Sensible heat production of laboratory rats in the MAEM depended on cage population density; sensible heat productions were 3.16 W/rat for MAEM-SD and 2.41 W/rat for MAEM-DD rats.

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Table 1. Mean room air temperatures (°C)

PHASE	LOCATION		
	INLET	CENTER	EXHAUST
REPLICATION 1			
AC	20.8±0.3	22.2±0.3	21.8±0.2
DC	21.5±0.5	22.3±0.3	22.1±0.4
RP	23.8±0.7	22.8±0.2	22.4±0.2
REPLICATION 2			
AC	22.2±0.5	22.6±0.3	22.3±0.3
DC	23.9±0.8	22.6±0.2	22.2±0.2
RP	21.4±0.3	22.6±0.1	22.3±0.2
REPLICATION 3			
AC	23.0±0.7	22.5±0.2	22.1±0.1
DC	21.4±0.2	22.6±0.1	22.4±0.1
RP	20.7±0.3	22.4±0.2	22.1±0.2
REPLICATION 4			
AC	21.3±0.2	22.6±0.1	22.4±0.1
DC	20.1±0.4	22.0±0.3	21.6±0.3
RP	20.2±0.3	21.8±0.2	21.5±0.2
REPLICATION 5			
AC	20.0±0.3	21.9±0.2	21.5±0.2
DC	20.1±0.3	21.8±0.2	21.5±0.2
RP	20.3±0.2	21.9±0.1	21.7±0.1
OVERALL	21.2±0.2	22.2±0.1	21.9±0.1

Table 2. Mean relative humidity (%) at the center of the room.

PHASE	0800 h	2000 h	MEAN
REPLICATION 1			
AC	50±3	51±2	50±2
DC	45±2	45±3	45±2
RP	48±4	48±3	49±3
REPLICATION 2			
AC	44±2	43±8	44±2
DC	56±4	52±3	55±3
RP	44±5	42±4	42±4
REPLICATION 3			
AC	54±4	51±3	52±3
DC	45±4	44±4	46±4
RP	59±3	54±4	56±2
REPLICATION 4			
AC	50±4	48±4	49±3
DC	63±4	58±5	61±4
RP	61±5	54±3	57±3
REPLICATION 5			
AC	61±4	58±4	59±3
DC	66±5	58±4	62±3
RP	61±1	64±2	63±2
OVERALL	54±1	53±1	54±1

Table 3. Mean cage air temperatures (°C).

PHASE	TREATMENT		
	PC	MAEM-SD	MAEM-DD
REPLICATION 1			
AC	23.6±0.2	23.8±0.2	23.5±0.2
DC	23.4±0.3	23.7±0.3	24.9±0.4
RP	23.5±0.2	24.1±0.3	24.2±0.2
REPLICATION 2			
AC	23.7±0.3	23.4±0.3	23.9±0.3
DC	23.3±0.2	24.0±0.2	24.7±0.3
RP	24.0±0.2	22.8±0.1	24.1±0.2
REPLICATION 3			
AC	22.9±0.2	23.0±0.1	22.8±0.1
DC	23.5±0.1	24.6±0.1	25.7±0.5
RP	24.1±0.2	22.8±0.2	23.3±0.2
REPLICATION 4			
AC	23.6±0.1	24.2±0.2	23.8±0.2
DC	23.3±0.3	23.4±0.4	25.0±0.3
RP	21.6±0.2	23.8±0.3	23.2±0.2
REPLICATION 5			
AC	23.2±0.2	23.2±0.2	23.3±0.2
DC	23.3±0.3	23.1±0.2	25.1±0.2
RP	20.5±0.1	23.7±0.1	23.2±0.1
OVERALL			
AC	23.4±0.2	23.5±0.2	23.5±0.2
DC	23.4±0.04	23.8±0.3	25.1±0.2
RP	23.3±0.5	23.6±0.2	23.6±0.2

Table 4. Mean air velocities (m/s).

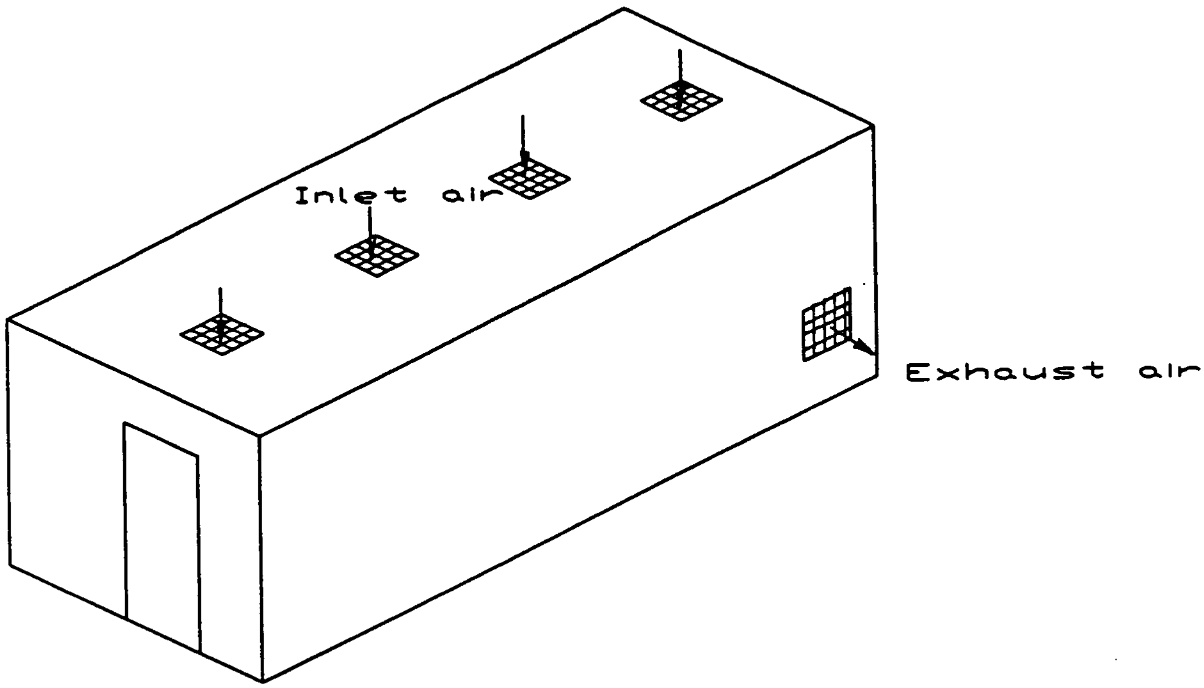
PHASE	TREATMENT					
	PC		MAEM-SD		MAEM-DD	
	0800 h	2000 h	0800 h	2000 h	0800 h	2000 h
REPLICATION 1						
AC	0.09±0.00	0.08±0.01	0.09±0.01	0.08±0.01	0.10±0.01	0.09±0.01
DC	0.07±0.00	0.07±0.01	0.13±0.00	0.14±0.01	0.13±0.00	0.14±0.01
RP	0.12±0.01	0.12±0.01	0.14±0.01	0.12±0.01	0.12±0.01	0.11±0.01
REPLICATION 2						
AC	0.08±0.01	0.09±0.01	0.08±0.01	0.08±0.01	0.07±0.01	0.07±0.01
DC	0.17±0.01	0.12±0.01	0.13±0.00	0.13±0.00	0.14±0.00	0.14±0.01
RP	0.08±0.00	0.09±0.01	0.08±0.01	0.09±0.01	0.09±0.00	0.10±0.01
REPLICATION 3						
AC	0.14±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.15±0.01	0.15±0.01
DC	0.08±0.01	0.10±0.01	0.13±0.00	0.13±0.01	0.13±0.00	0.12±0.00
RP	0.12±0.01	0.13±0.01	0.10±0.01	0.10±0.01	0.09±0.01	0.09±0.00
REPLICATION 4						
AC	0.09±0.00	0.09±0.00	0.09±0.01	0.09±0.00	0.10±0.00	0.10±0.00
DC	0.09±0.01	0.10±0.01	0.13±0.01	0.14±0.00	0.12±0.00	0.13±0.00
RP	0.10±0.01	0.10±0.00	0.09±0.01	0.09±0.01	0.10±0.01	0.10±0.01
REPLICATION 5						
AC	0.08±0.00	0.08±0.00	0.10±0.00	0.10±0.00	0.09±0.00	0.09±0.01
DC	0.09±0.00	0.08±0.00	0.12±0.00	0.13±0.00	0.12±0.00	0.13±0.00
RP	0.12±0.02	0.11±0.01	0.18±0.02	0.17±0.02	0.12±0.02	0.11±0.02
OVERALL						
AC	0.10±0.00	0.10±0.01	0.10±0.01	0.10±0.01	0.10±0.01	0.10±0.01
DC	0.10±0.02	0.10±0.01	0.13±0.00	0.12±0.00	0.13±0.00	0.13±0.00
RP	0.11±0.01	0.11±0.01	0.12±0.02	0.12±0.02	0.11±0.01	0.10±0.00

Table 5. Mean noise levels (dBA).

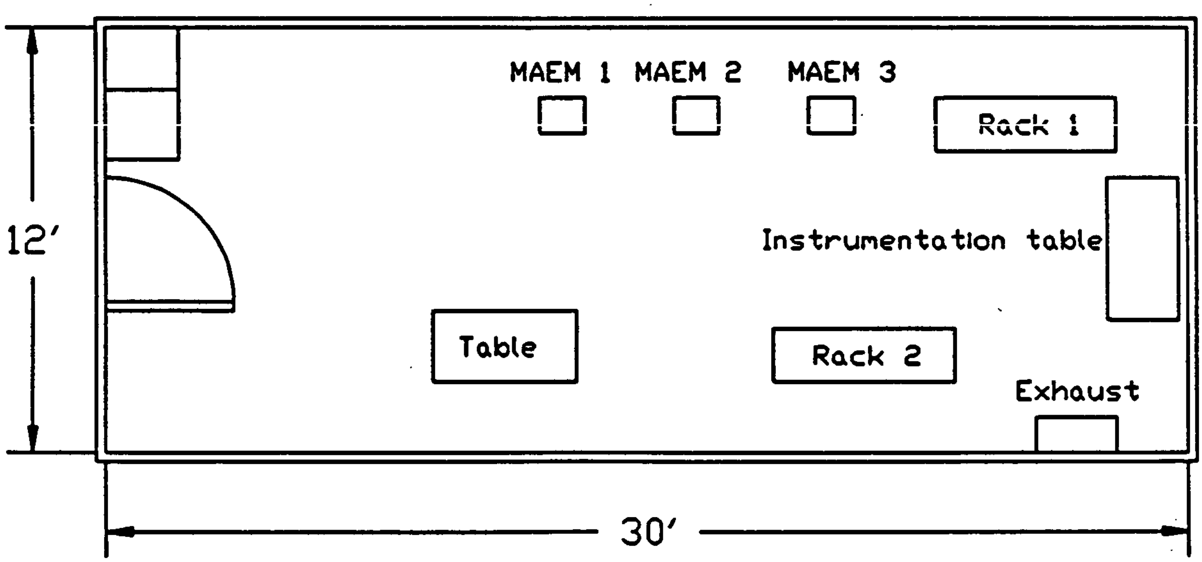
PHASE	TREATMENT					
	PC		MAEM-SD		MAEM-DD	
	0800 h	2000 h	0800 h	2000 h	0800 h	2000 h
REPLICATION 1						
AC	54±1	55±2	54±1	56±2	54±1	56±2
DC	50±3	53±0	50±0	51±0	51±0	53±1
RP	60±2	63±2	60±2	62±1	60±2	62±1
REPLICATION 2						
AC	53±1	55±1	54±1	56±2	54±1	55±1
DC	63±1	65±0	56±0	58±1	57±1	62±2
RP	54±0	55±1	53±0	55±1	54±0	55±1
REPLICATION 3						
AC	61±1	63±1	61±1	63±1	60±1	63±1
DC	53±0	53±0	50±0	51±0	51±1	54±1
RP	51±0	52±0	52±0	52±0	52±0	53±0
REPLICATION 4						
AC	52±0	52±0	52±0	52±0	52±0	52±0
DC	52±0	52±0	51±0	53±0	50±0	56±1
RP	56±1	56±1	57±1	56±1	57±1	56±1
REPLICATION 5						
AC	53±0	53±0	52±0	53±0	52±0	52±0
DC	57±1	58±1	52±1	51±0	52±2	53±1
RP	58±0	59±1	59±1	59±0	58±1	58±1
OVERALL						
AC	55±2	56±2	55±2	56±2	54±1	56±2
DC	55±2	56±2	52±1	53±1	52±1	56±2
RP	56±2	56±2	56±2	57±2	56±1	57±2

Table 6. Mean light intensity levels (lux).

PHASE	TREATMENT					
	PC		MAEM-SD		MAEM-DD	
	0800 h	2000 h	0800 h	2000 h	0800 h	2000 h
REPLICATION 1						
AC	49±2	9±1	48±1	11±1	45±1	8±1
DC	52±1	5±0	215±3	3±0	240±1	4±0
RP	58±1	4±0	50±1	4±0	68±2	4±0
REPLICATION 2						
AC	41±1	3±0	41±1	3±0	40±1	3±0
DC	39±1	3±0	205±1	4±0	235±1	4±0
RP	32±1	3±0	36±1	3±0	42±2	3±0
REPLICATION 3						
AC	32±1	3±0	31±1	3±0	47±1	3±0
DC	31±1	3±0	210±5	4±0	225±6	4±0
RP	30±2	3±0	30±2	3±0	35±3	3±0
REPLICATION 4						
AC	59±2	4±0	42±1	4±0	46±1	4±0
DC	53±2	4±0	251±5	4±0	226±8	4±0
RP	54±2	4±0	47±2	5±0	54±2	4±0
REPLICATION 5						
AC	35±1	3±0	36±1	3±0	37±2	3±0
DC	38±1	3±0	236±5	4±0	200±7	4±0
RP	35±2	4±0	43±2	3±0	37±1	4±0
OVERALL						
AC	43±5	4±1	40±3	5±2	43±2	4±1
DC	43±4	4±0	223±9	4±0	225±7	4±0
RP	42±6	4±0	41±4	4±0	47±6	4±0

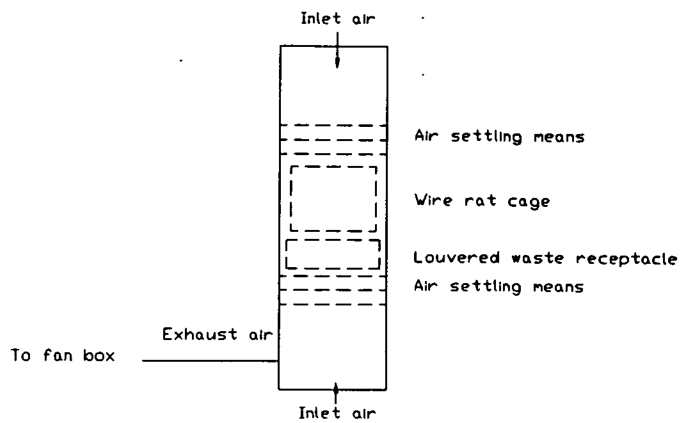


(a)

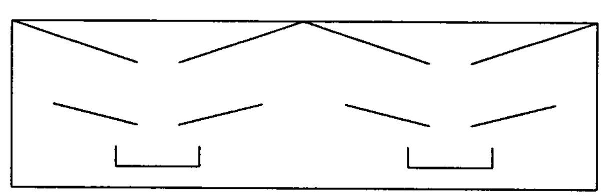


(b)

Figure 1. The laboratory room: (a) sketch showing the location of the air inlets and the exhaust outlet and (b) layout showing the cage arrangements.



(a)



(b)

Figure 2. Schematic diagram of (a) the mock animal enclosure module and (b) the louvered waste receptacle.

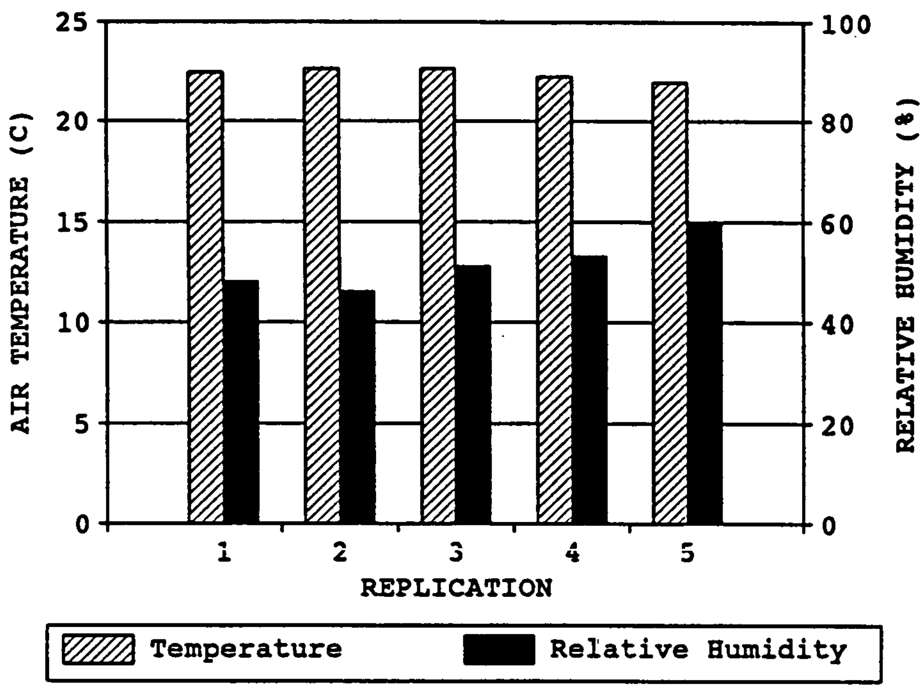


Figure 3. Mean room air temperatures and relative humidities.
SEMs are given in Tables 1 and 2.

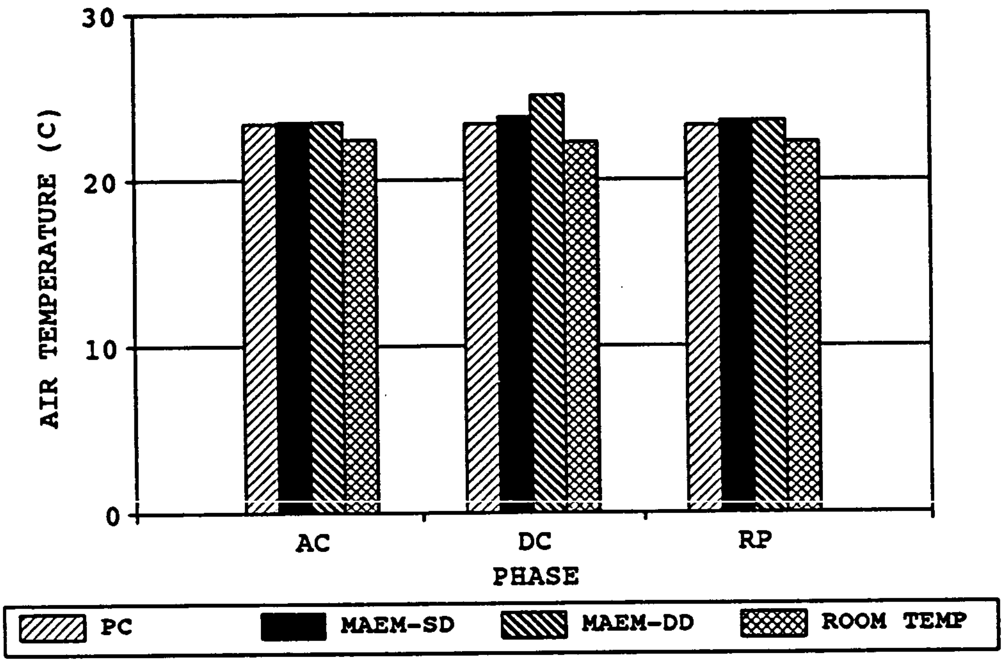


Figure 4. Mean cage air temperatures.
SEMs are given in Table 3.

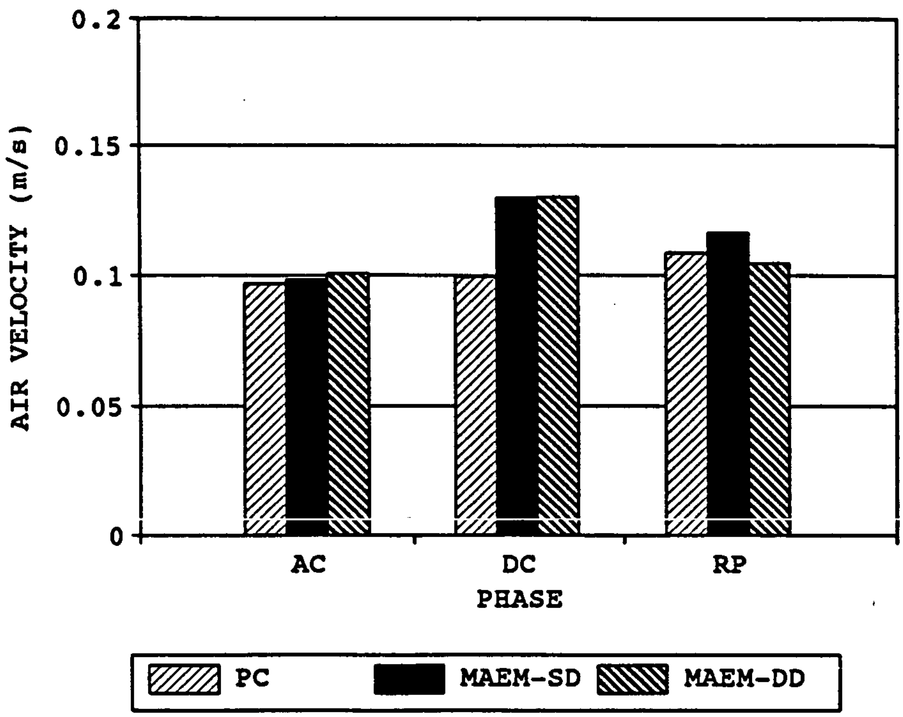


Figure 5. Mean air velocities approaching the cages.
SEMs are given in Table 4

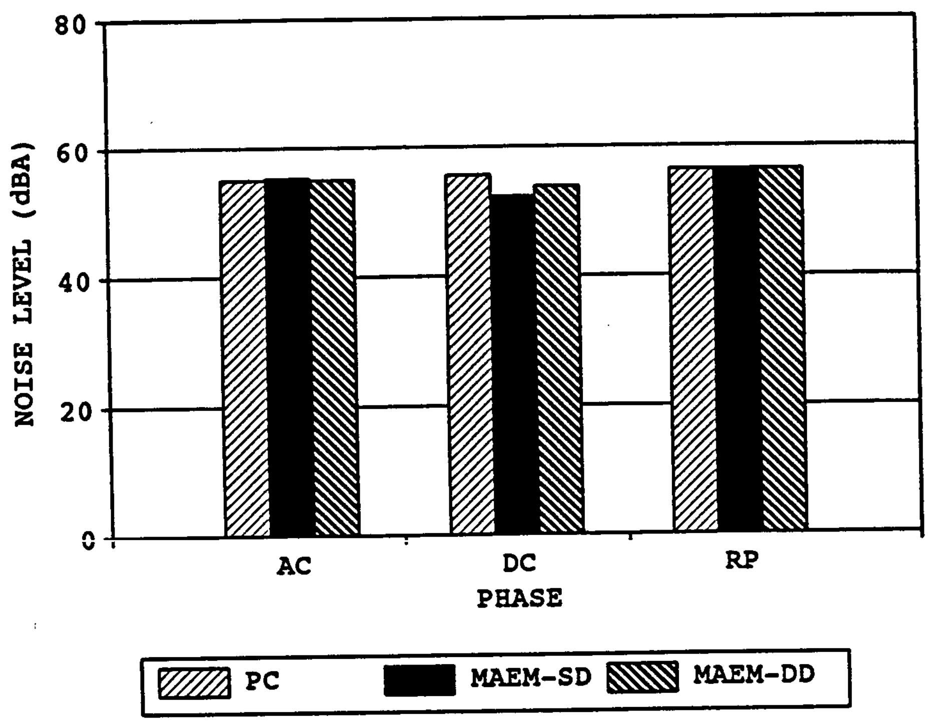


Figure 6. Mean noise levels within the cages.
SEMs are given in Table 5.

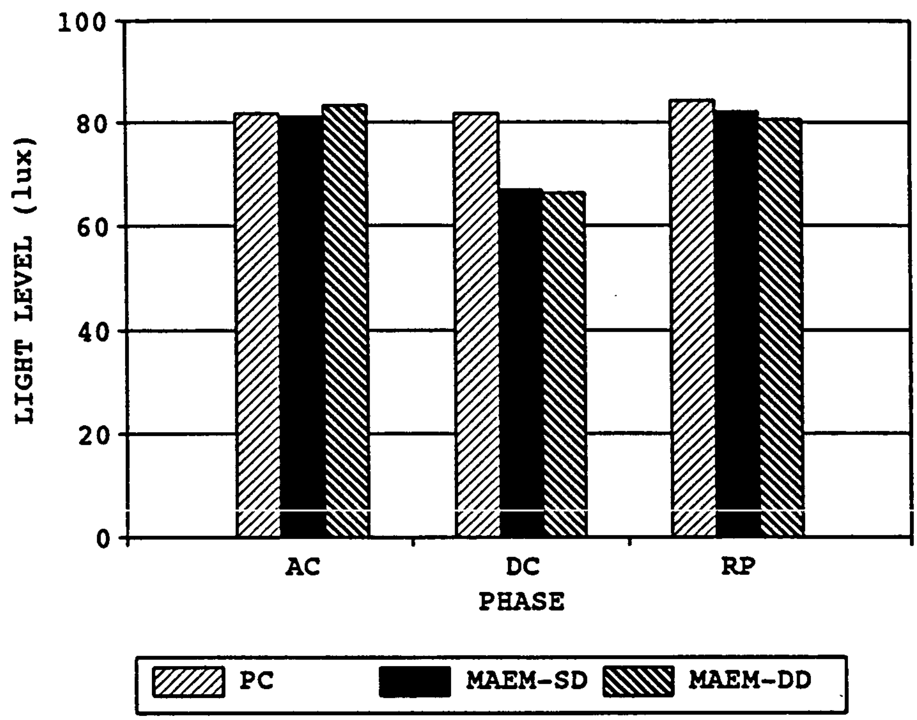


Figure 7. Mean light levels within the cages during "light" hours.
SEMs are given in Table 6

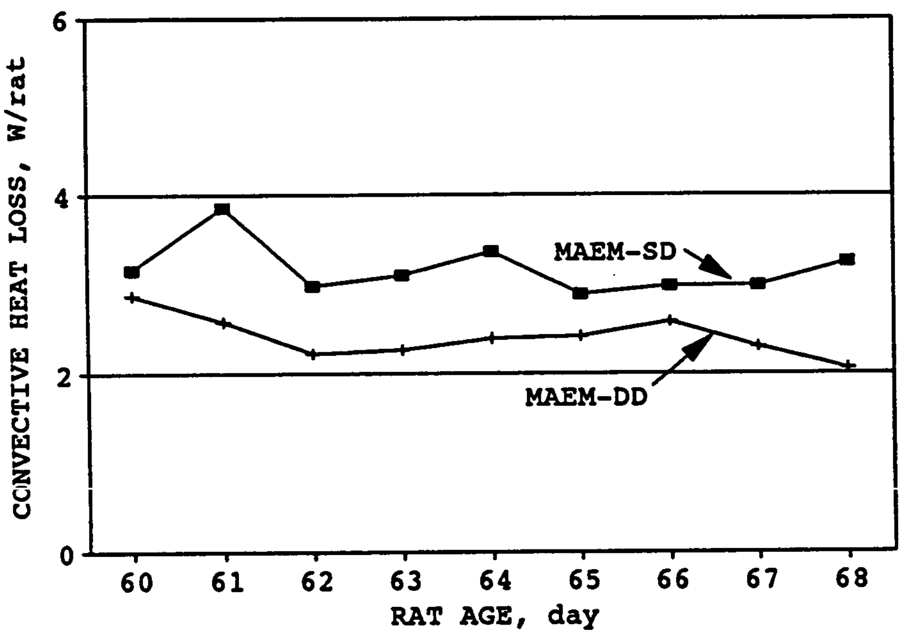


Figure 8. Mean convective heat losses of laboratory rats in the MAEM.

Effect of Double Density Caging during Space Shuttle Transport of Laboratory Rats on Food and Water Intake, Weight Changes, Rate of Gain, Tissue Weights, and Physical Appearance

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Materials and Methods

Food and water intake, weight changes, and rate of gain

To mimic conditions in the space shuttle, rats were fed and watered using systems similar to those used in the Animal Enclosure Module (AEM). Each cage had its own water system. In polycarbonate cages (PC) the watering system consisted of two inverted 100 ml graduated cylinders plugged with a rubber stopper containing a watering tube. The two watering tubes were connected by plastic tubing to a Lixit waterer placed through the wire top of the PC cage within reach of the rats. The MAEM had a waterer with four Lixits built into the cage like that of the AEM. Water was supplied to this system in a similar fashion as the PC cages. After the first replication, the 100 ml graduated cylinders were replaced with one 250 ml graduated cylinder in the MAEM-SD and two 250 ml graduated cylinders in the MAEM-DD.

Rats in all treatments were fed a diet of prepared food bars for all three phases. Food bars were wired to metal plates and attached to the sides of the cage. After replicate 1 of the AC phase, we determined that a cage of four rats consumed nearly 100 g of food bar daily. Each food bar weighed approximately 200 g and food was provided for each phase as follows: AC: each cage received 7 food bars; DC: PC and MAEM-SD treatments were each supplied with 5 food bars while MAEM-DD had 10; and RP: each cage received 5 food bars. If the rats consumed all their food before the end of the phrase, additional food bars were provided.

Feed was weighed to the nearest 0.1 g prior to being placed in a cage before each phase and again at the end of a phase. In addition, feed was weighed at days 2 and 5 during DC and at day 5 during RP. Water intake was measured to the nearest 0.5 ml, daily. Feed and water intakes were calculated as cage means.

Rats were weighed individually to the nearest 0.1 g at the beginning and end of each phase. Additionally, rats were weighed at days 2 and 5 in DC phase and rats in RP phase were weighed at day 5. Percent weight change was calculated as a cage mean. The gain to feed values were calculated by dividing the mean daily weight gain (g) per rat by the mean daily feed intake per rat for each of the three phases.

Cage means were analyzed for replicate and treatment effects using the GLM procedure of SAS (1985). Feed and water intake for MAEM-DD treatment were adjusted to reflect a four rat cage so direct comparisons could be made. Each cage of rats was an experimental unit. Thus, rats within a cage contributed to the mean of the cage, which was used as one data point in calculating treatment means. A randomized complete block analysis was used to investigate the effect of housing systems on food and water intake, gain to feed values, body weight, and tissue weight responses of rats. The model included replication as blocks, housing systems as treatments, and replication x treatment; replication x treatment served as the error term.

Tissue weights: adrenals, thymus, GI tract, and testes

At the end of the DC and RP phases of each replication, 12 rats were sacrificed; 4 from MAEM-DD, 4 from MAEM-SD, and 4 from PC cages. The animals were necropsied and adrenals, thymus and testes were removed, trimmed of excess fat or tissue, weighed, and placed in formalin jars. The GI tract was sutured at the opening to the stomach and at the colon rectal area and then removed. After removal, the tract was weighed, flushed out, and weighed again, empty. Finally the GI tract was preserved in formalin.

Tissue weights were calculated as a percentage of total body weight and means calculated on a cage basis. Data were analyzed for treatment and replication effects using the GLM procedure of SAS (1985).

Physical appearance

Rats were evaluated for physical appearance at the end of DC and RP phases. Before the animals were sacrificed at the end of DC phase, both MAEM and PC rats were evaluated for general physical appearance on a cage basis. Rats were ranked from best to worst based on the overall appearance of hair coat, eyes, ears, and nose. In addition, rats that were to continue on into the RP were ranked individually for these measurements. Each rat was placed in a separate PC cage and ranked for condition of hair coat first, ears second, and finally eyes and nose. Judgement of coat condition was based on color and degree of mattedness, ears were based on cleanliness and color, and eyes and nose were based on color and the appearance of any discharges. Rats were ranked from best to worst condition for each category.

At the end of RP, general physical appearances were checked on a cage basis and rats were ranked on a cage basis from best to worst overall appearance.

Ranks were analyzed using normal order statistics in a hierarchical analysis of variance for the individual physical evaluation. Cage ranks for DC were analyzed via the Kruskal-Wallis H test and cage ranks for recovery were analyzed via Friedman's Randomized Blocks.

Results and Discussion

Food and water intake, weight changes, and rate of gain

LSmeans for daily feed and water intake for each cage treatment during the three phases are presented in Table 1. During AC, replicates were significantly different for food intake. Replicates 1 and 2 had lower intakes than replicates 3, 4, and 5 ($P<0.005$) and replicate 4 was lower than replicate 5 ($P<0.05$). During DC, MAEM-SD food intake was higher than both MAEM-DD and PC ($P<0.05$) and water intake was lowest for PC ($P<0.005$). Leakage from the waterers was a problem in both cage types and may have had an effect on measured intakes. In MAEMs, rats laid under the waterers pressing up against the Lixits causing leaks. Replicates were not significantly different. No significant differences occurred due to replicate or treatment for food and water intake during RP phase.

Percent body weight changes by treatment for DC are reported in Table 2 and compared in Fig. 1. MAEM-DD had lower ($P<0.05$) weight gains than either MAEM-SD or PC during the first five days of DC. In the last five days of DC, percent body weight changes were not significantly different. Over the total DC phase, MAEM-DD had lower percent body weight gains ($P<0.05$) than PC, but PC and MAEM-SD were not significantly different. Among replicates, replicate 4 had lowest overall weight gains for DC phase.

No significant differences in percent body weight change were found for days 0-5 and total of RP phase (Table 3 and Fig. 2). Percent body weight changes were higher ($P<0.05$) for both MAEM-DD and MAEM-SD rats as compared to PC rats for days 6-10. A problem with the scale used to weigh the rats in replication 3 may have caused a measurement error in the day 6-10 weight gains for MAEM-SD rats so this data was excluded in the analysis.

Gain to feed values for DC phase were significantly higher ($P<0.05$) for rats in the PCs as compared to both MAEM-SD and MAEM-DD (Table 4). Rats in replicate 4 had lower gain to feed values than all other replicates ($P<0.05$). No significant effects of treatment or replicate were found for gain to feed values for RP phase.

Rats in MAEM-SD consumed more food than MAEM-DD and PC rats during DC phase. Doubling the density may have caused a decreased food intake due to crowding. Rats in MAEM-DD had lower percent weight gains compared to PC and MAEM-SD rats which may also indicate that crowding may have an effect during DC. The effect on weight gain appears greatest within the first two days of DC especially in the MAEM-DD treatment. As seen in Table 2, the percent body weight change for the PC and MAEM-SD rats was approximately two percentage points higher than the MAEM-DD rats during the first two days of DC. After the first five days, no significant difference was found among the three treatments (Table 2), which suggests that the stress may be short term. Gain to feed values reflect a cage type effect with MAEM-DD and MAEM-SD significantly lower in gain to feed conversion than PC. Replication effects probably were due to differences in initial weights of rats at the beginning of the phases. Table 6 shows initial rat weights (average per rat weight for each cage) at beginning of Acclimation phase.

Although there appears to be effects of treatment during the DC phase, rats seemed to be able to recover, as evidenced by lack of significant effects of treatment during RP period for food and water intake and gain to feed values. The higher percent weight increase for MAEM-DD and MAEM-SD rats in the latter half of RP may indicate a compensatory growth.

Tissue weights: adrenals, thymus, GI tract, and testes

LSmeans for tissue weights calculated as a percentage of body weight for each treatment are reported in Table 5 for DC and RP phases. GI tract, testes, thymus and adrenal weights for DC and RP phases are compared in Figs. 3, 4, 5 and 6, respectively.

Adrenal weights in MAEM-DD and MAEM-SD were significantly higher ($P<0.005$) than PC for DC. In RP, adrenal weights were significantly lower for MAEM-DD and MAEM-SD rats compared to PC rats. A replicate effect was found for adrenal and thymus weights during DC and for thymus weights during RP.

Stress may affect tissue weights of an animal due to the physiologic processes. Differences in adrenal weights may be indicative of stress due to both cage type and increased density. Replication effects occurring in thymus and adrenal weights were probably due to trimming of fat and excess tissue around the gland.

Physical appearance

There were significant differences ($P<0.001$) in physical appearance among cage types and densities during DC. Individual PC rats were ranked best for physical appearance (a combination of hair coat, ears, and eyes and nose rankings), followed by MAEM-DD rats, and finally MAEM-SD rats. Analysis of cage rankings for DC revealed PC cages had the best physical appearance, with MAEM-SD rats second, and MAEM-DD last ($P<0.05$).

No significant differences in physical appearance were evident in RP.

Differences among treatments in DC suggest that both cage type and density have a detrimental effect on the physical appearance of the rat. The effect does not appear to be long term as rats showed no significant differences in RP.

References

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Zar, Jerrold H. 1984. Biostatistical Analysis. Prentice-Hall, New Jersey.

Acknowledgement

I would like to thank Dr. A. W. Ghent, University of Illinois, for his assistance with the statistical analysis of the physical appearance data.

Table 1. Daily food and water intake for cage treatment in AC, DC, and RP. LSmeans ± SEM for cage reported, n=5.

Acclimation				
	Food intake (g)	93.7 ± 0.6		
	Water intake (ml)	99.6 ± 2.8		
		MAEM-DD†	MAEM-SD	PC
Density Challenge				
	Food intake (g)	98.3 ± 0.9^{a}	108.5 ± 2.7^{b}	94.5 ± 1.4^{a}
	Water intake (ml)	172.7 ± 11.6^{c}	151.5 ± 3.2^{c}	101.5 ± 4.2^{d}
Recovery				
	Food intake (g)	101.7 ± 1.5	104.3 ± 3.5	106.1 ± 4.1
	Water intake (ml)	99.6 ± 7.4	105.4 ± 5.7	107.3 ± 8.7

†Adjusted to reflect a four rat cage.

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

[c,d]Means in the same row with different superscripts differ ($P=0.005$).

Rows without superscripts are not significantly different

Table 2. Mean percent body weight change by cage treatment for DC. Percent weight change from previous weigh date. LSmeans $\pm$ SEM for cage reported, n=5.

	Percent Weight Change		
	MAEM-DD	MAEM-SD	PC
Days 0-2	1.14 ± 0.56^{a}	3.14 ± 0.86^{b}	3.40 ± 0.80^{b}
Days 3-5	3.90 ± 0.51^{a}	4.44 ± 0.47^{b}	4.89 ± 0.50^{b}
Days 6-10	5.38 ± 0.88	4.70 ± 1.41	6.77 ± 0.64
Total	10.70 ± 0.91^{a}	12.81 ± 2.28^{ab}	15.79 ± 0.94^{b}

^{a,b}Means in the same row with different superscript letters differ ($P<0.05$).
Rows without superscripts are not significantly different.

Table 3. Mean percent body weight change by cage treatment for RP. Percent weight change from previous weigh date. LSmeans $\pm$ SEM for cage reported, n=5.

	Percent Weight Change		
	MAEM-DD	MAEM-SD	PC
Days 0-5	7.33 $\pm$ 0.56	8.25 $\pm$ 1.71	7.24 $\pm$ 0.57
Days 6-10	5.47 $\pm$ 0.74[a]	4.27 $\pm$ 0.50[a*]	2.59 $\pm$ 0.72[b]
Total	13.18 $\pm$ 1.26	13.18 $\pm$ 2.74	10.01 $\pm$ 0.85

[a,b]Means in the same row with different superscripts differ ($P<0.05$).
*n=4 for MAEM-SD due to errors in weight measurements in replication 3.

Table 4. Daily gain to feed values for cage treatments in AC, DC, and RP. LSmeans $\pm$ SEM for cage reported.

	Gain to Feed Values		
Acclimation	0.284 + 0.003		
	MAEM-DD	MAEM-SD	PC
Density Challenge	0.125 ± 0.010^a	0.134 ± 0.019^a	0.190 ± 0.010^b
Recovery	0.163 ± 0.013	0.150 ± 0.019	0.154 ± 0.031

[a,b]Means in the same row with different superscripts differ ($P<0.05$).
Rows without superscripts are not significantly different.

Table 5. GI tract (empty), testes, thymus and adrenal gland weight as a percent of body weight by cage treatment for DC and RP.
LSmeans $\pm$ SEM for cage reported, n=5.

	MAEM-DD	MAEM-SD	PC
Density Challenge			
GI	3.51 $\pm$ 0.12	3.76 $\pm$ 0.08	3.62 $\pm$ 0.06
Testes	1.07 $\pm$ 0.03	1.11 $\pm$ 0.03	1.08 $\pm$ 0.03
Thymus	0.20 $\pm$ 0.016	0.20 $\pm$ 0.009	0.21 $\pm$ 0.009
Adrenals	0.016 $\pm$ 0.002[a]	0.018 $\pm$ 0.002[a]	0.014 $\pm$ 0.002[b]
Recovery			
GI	3.30 $\pm$ 0.03	3.42 $\pm$ 0.07	3.39 $\pm$ 0.05
Testes	0.99 $\pm$ 0.02	0.98 $\pm$ 0.05	1.01 $\pm$ 0.01
Thymus	0.16 $\pm$ 0.005	0.17 $\pm$ 0.005	0.16 $\pm$ 0.008
Adrenals	0.012 $\pm$ 0.0007[c]	0.012 $\pm$ 0.0003[c]	0.014 $\pm$ 0.0006[d]

[a,b]Means in the same row with different superscripts differ ($P<0.005$).
[c,d]Means in the same row with different superscripts differ ($P<0.05$).
Rows without superscripts are not significantly different.

Table 6. Starting rat weights on a per rate basis at the beginning of the Acclimation period for each replication. Cage LS means ± SEM are reported.

REPLICATION		STARTING WT. (g)
ONE		
	Cage 1	195.7 ± 4.0*
	Cage 2	195.5 ± 3.8
	Cage 3	195.7 ± 3.5
	Cage 4	195.3 ± 2.5
	Cage 5	195.1 ± 2.6
	Cage 6	195.0 ± 2.6
	Cage 7	195.1 ± 2.5
TWO		
	Cage 1	184.9 ± 5.8
	Cage 2	185.4 ± 4.5
	Cage 3	185.6 ± 3.8
	Cage 4	185.3 ± 3.2
	Cage 5	185.7 ± 3.3*
	Cage 6	185.9 ± 3.1
	Cage 7	186.2 ± 3.0
THREE		
	Cage 1	195.0 ± 4.3
	Cage 2	195.1 ± 3.7
	Cage 3	195.2 ± 3.5
	Cage 4	195.6 ± 3.0*
	Cage 5	195.2 ± 2.6
	Cage 6	195.3 ± 2.4
	Cage 7	194.7 ± 2.1
FOUR		
	Cage 1	194.6 + 4.8
	Cage 2	194.5 ± 4.6*
	Cage 3	194.3 ± 3.8
	Cage 4	194.5 ± 3.3
	Cage 5	194.2 ± 2.9
	Cage 6	194.3 ± 2.8
	Cage 7	194.0 ± 2.7
FIVE		
	Cage 1	198.0 ± 5.4
	Cage 2	197.8 ± 4.6
	Cage 3	197.2 ± 3.8
	Cage 4	197.0 ± 3.6
	Cage 5	197.2 ± 2.7
	Cage 6	196.8 ± 2.4*
	Cage 7	197.3 ± 2.3

* These cages were not used in Density challenge or Recovery phases.

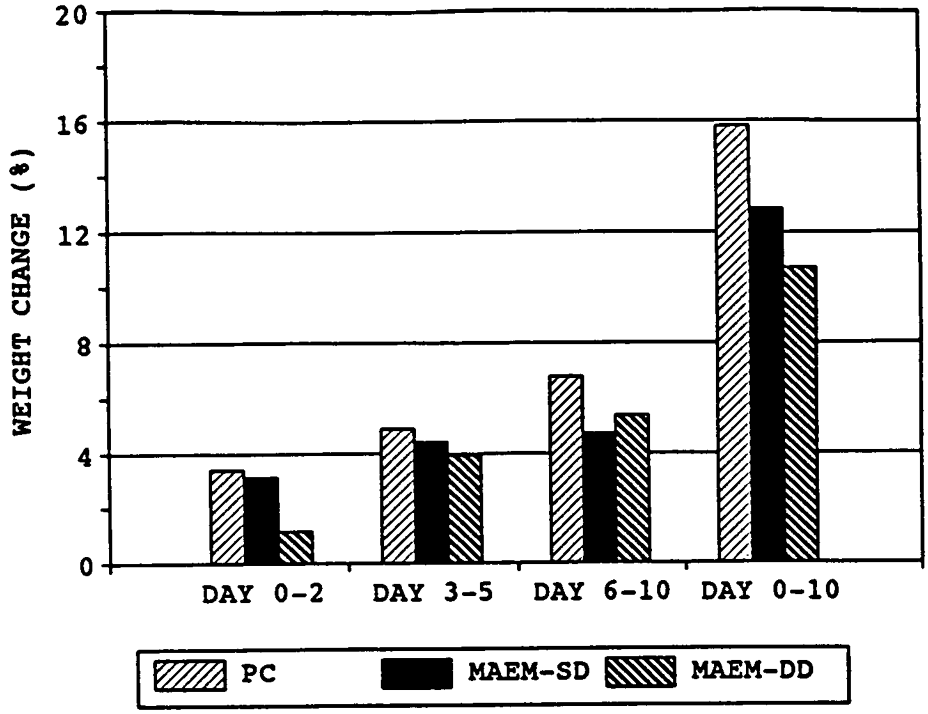

Fig. 1. Percent body weight change for DC.
SEMs are given in Table 2.

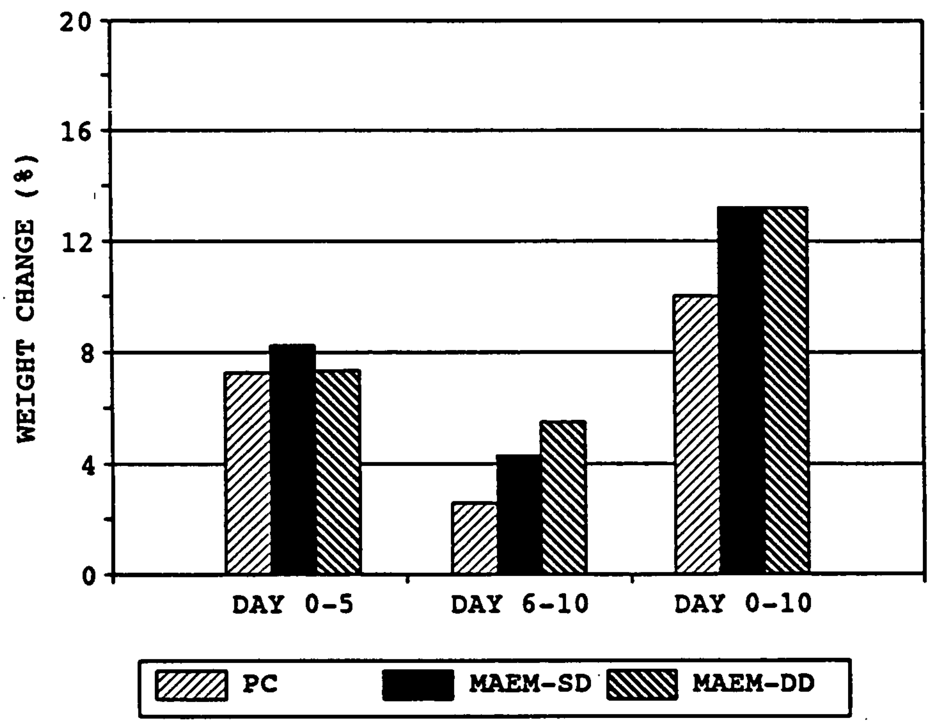

Fig. 2. Percent body weight change for RP.
SEMs are given in Table 3.

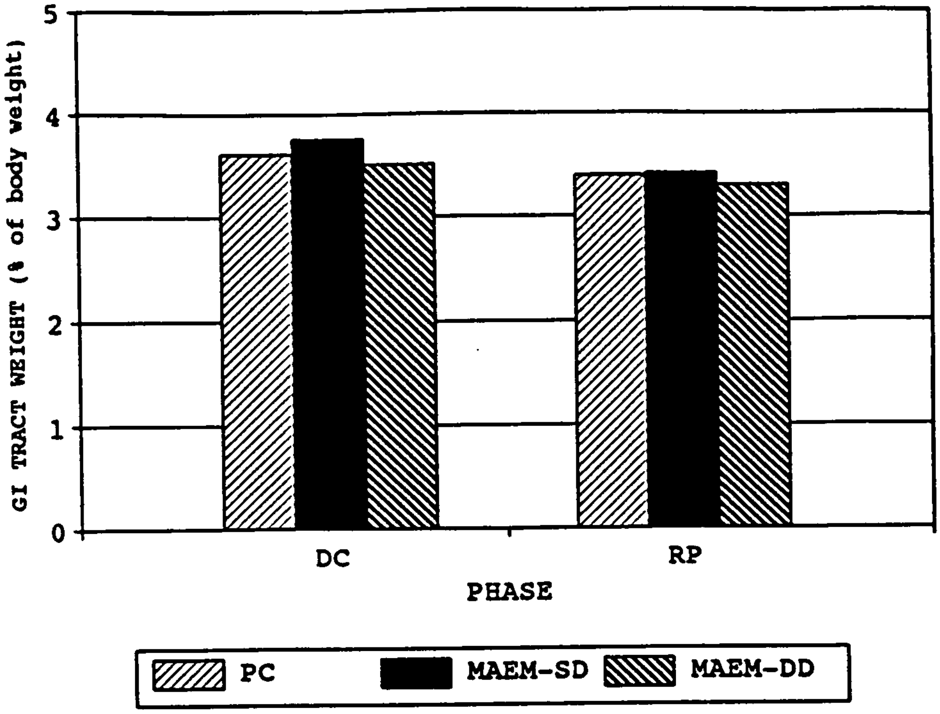

Fig. 3. GI tract (empty) weight as a percent of total body weight for DC and RP. SEMs are given in Table 5.

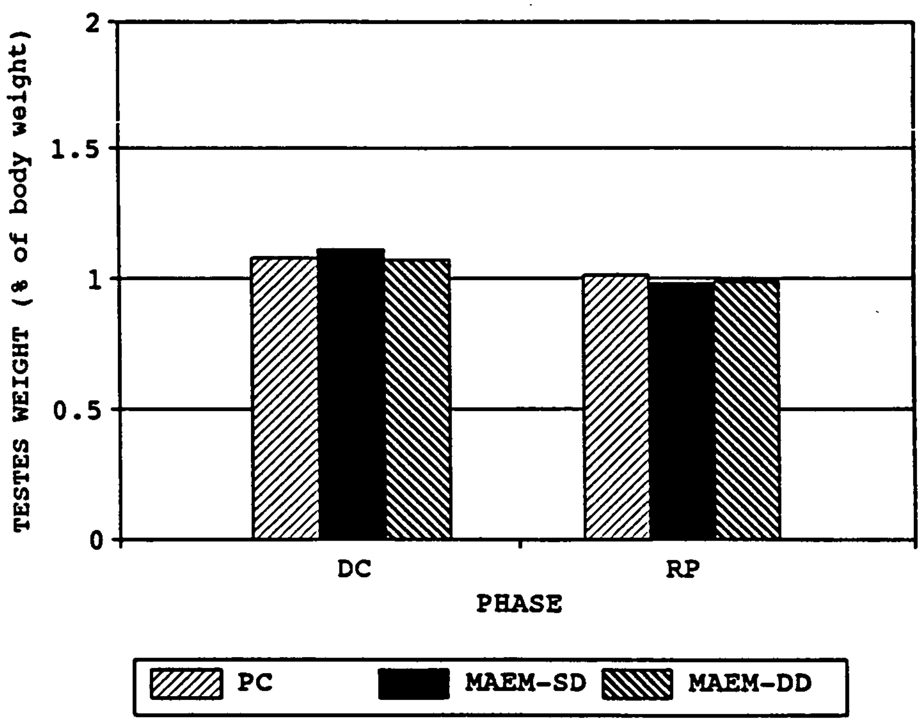

Fig. 4. Testes weight as a percent of body weight for DC and RP. SEMs are given in Table 5.

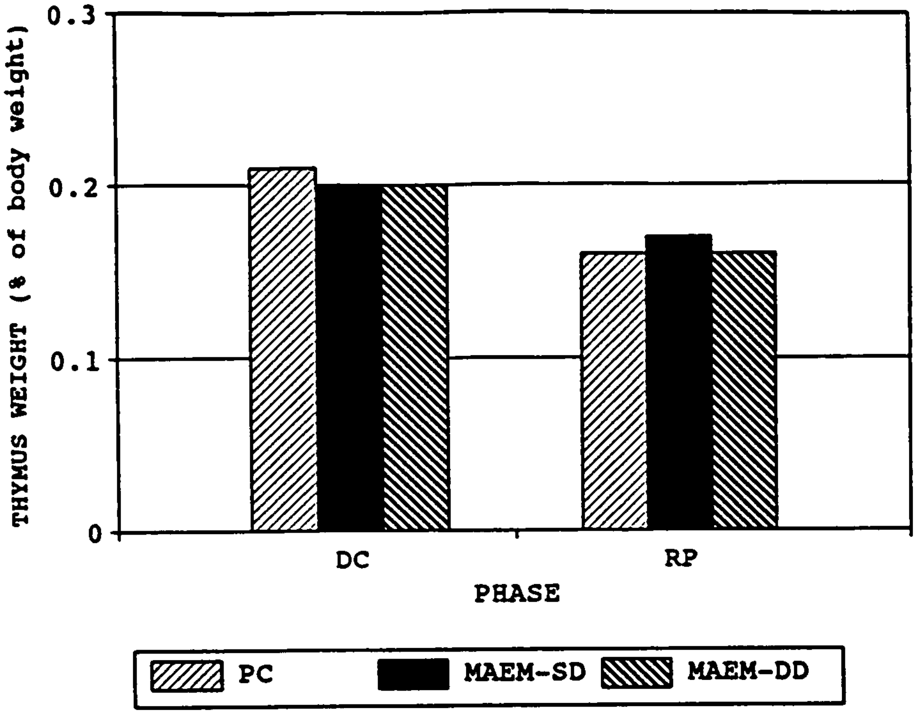

Fig. 5. Thymus weight as a percent of body weight for DC and RP. SEMs are given in Table 5.

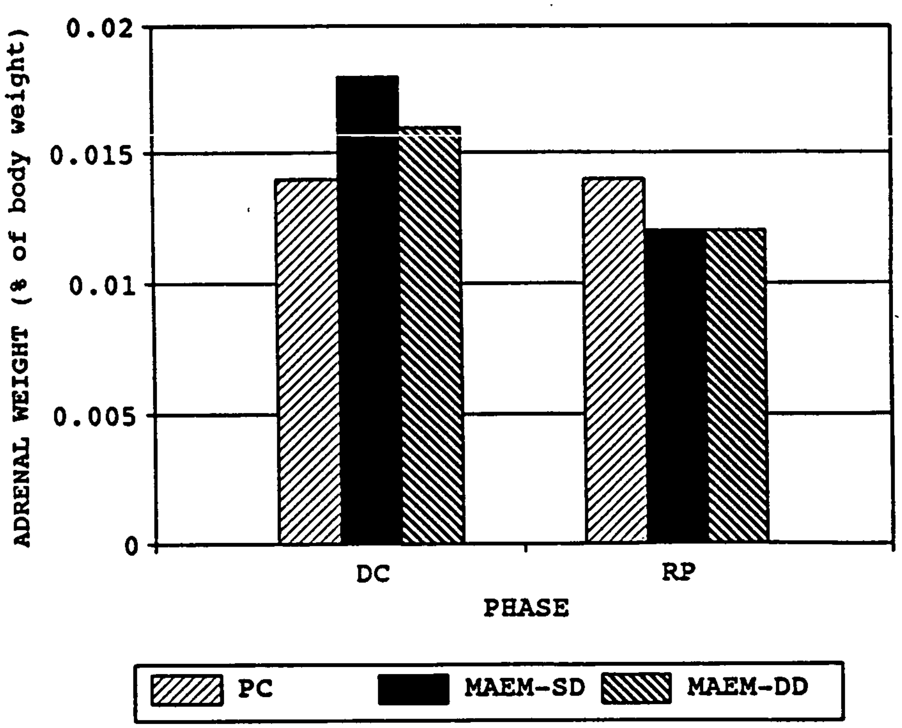

Fig. 6. Adrenal weight as a percent of body weight for DC and RP. SEMs are given in Table 5.

Effect of Double Density Housing in Mock Animal Enclosure Modules (MAEM) on Several Physiologic and Immunologic Responses of Male Sprague Dawley Rats

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Materials and Methods

Lectin-induced lymphocyte proliferation

Cell suspensions prepared from spleens and erythrocytes were lysed with 0.83% NH_4Cl in 0.1% $KHCO_3$/0.01 mM EDTA. Splenocytes were suspended at 5 x 10^6 cells/mL in RPMI 1640 medium supplemented with 5% fetal calf serum, 100 units of penicillin/mL, 100 μg streptomycin/mL and 24 mM $NaHCO_3$. Aliquots consisting of 100 μL of cells and 100 μL of various concentrations of Concanavalin A (Con A) were plated out in 96-well plates (each sample was done in triplicate) and incubated at 39°C in 7% CO_2 for 72 h. One μCi of [methyl-3H] thymidine was added to each well and incubated for an additional 18 h. Cells were harvested onto glass fiber disks. Disks were dried with 70% ethanol and placed in an 80°C oven for 15 min. Scintillation cocktail of toluene-Omniflour was added and radioactivity was measured with a Beckman LS 5801 liquid scintillation counter. Lymphocyte proliferation was chosen because it is correlated negatively to circulating corticosterone levels.

Corticosterone

Corticosterone (the major rat glucocorticoid) concentrations were evaluated from plasma obtained during sacrifice. Values were determined by using the RSL ^{125}I Corticosterone Kit for rats and mice (ICN Biomedicals Inc., Costa Mesa, CA). Radioactivity was measured with a Beckman gamma counter. Circulating corticosterone was measured because it is a classic indicator of stress. To avoid the circadian rhythm of corticosterone release, with the highest concentrations being reported between 1600 h and 2200 h, blood samples were collected at 0900 h. After performing preliminary work on the effects of different euthanasia procedures on corticosterone values, it was discovered that using CO_2 anesthesia prior to decapitation provided values closest to baseline values thereby reducing any false readings as a result of any extraneous stressors such as handling. Handling variables were further reduced by anesthetizing the rats in their respective cages.

Total plasma protein

Plasma protein was evaluated from samples obtained during sacrifice. Values were determined by using a Total Protein assay (Sigma Diagnostics, St. Louis MO).

Leukocyte differential counts

Blood smears were made at the time of sacrifice, stained and analyzed with special attention to neutrophils and lymphocytes. The neutrophil:lymphocyte ratio is a classic indicator of stress and is correlated positively to circulating corticosterone levels.

Hematocrit

Packed cell volume for each rat was obtained as the mean of three replicate hematocrit readings.

Statistic Analysis

A general linear models procedure, using the statistical analyses system (SAS), was performed to determine if any treatment and replication differences were present. Each cage of rats was an experimental unit. Thus, rats within a cage contributed to the mean of the cage, which was used as one data point in calculating treatment means. A randomized complete block analysis was used to investigate the effect of housing systems on immunologic and physiologic responses of rats. The model included replication as blocks, housing systems as treatments, and replication x treatment; replication x treatment served as the error term.

Results and Discussion

Lectin-induced proliferation for DC and RP are presented in Table 1 as lymphocyte incorporation of tritiated labeled thymidine. During the DC phase, rats which were exposed to MAEM-DD had a higher degree of responsiveness than did MAEM-SD and PC at all concentrations of mitogen (Figure 1). MAEM-DD rats also expressed the highest level of corticosterone during DC (Figure 3). However, none of these differences were statistically significant. High levels of corticosterone normally would result in decreased responsiveness to mitogen.

Glucocorticoids inhibit the expression of Interleukin-2 (IL-2) and/or the receptor for IL-2 which are necessary for lymphocyte proliferation. Although MAEM-DD had the highest level of corticosterone, 45.21 ng/mL is within baseline range. This would explain why we did not see a decrease in responsiveness to mitogen. However, these immunosuppressive effects are reinforced by the results obtained during RP. MAEM-SD rats exhibited the lowest level of corticosterone and the highest degree of responsiveness to mitogen stimulation at concentrations of 6 μg/mL, 3μg/mL and 1.5μg/mL. There were no differences ($P>0.05$) among treatments at all concentrations. However, a difference ($P<0.05$) did exist between replications at concentrations of 3 μg/mL and 1.5 μg/mL. Differences were among replications 1 and 2, 2 and 3, 2 and 5 and 1 and 3, 2 and 3, 2 and 5, 3 and 4, 4 and 5, respectively. Figure 2 represents the rats that continued on into RP. MAEM-DD and PC rats appeared to be equally responsive at all concentrations. There were no differences ($P>0.05$) among treatments at all concentrations. However, a significant difference ($P < 0.05$) existed between replications at 24 μg/mL (1 and 2, 1 and 3, 1 and 4, 1 and 5, 2 and 3, 2 and 4, and 2 and 5), 12 μg/mL (1 and 3, 1 and 4, 1 and 5, 2 and 4, and 2 and 5), 6 μg/mL (1 and 4 and 1 and 5), 3 μg/mL (1 and 2, 1 and 4, 1 and 5, and 3 and 4) and 1.5 μg/mL (1 and 4, 3 and 4, and 4 and 5).

Plasma corticosterone levels for DC and RP are presented in Table 2. During DC, MAEM-DD rats had a 50% higher corticosterone level than MAEM-SD and PC rats. This difference was not significant ($P > 0.05$). MAEM-DD rats that went on to RP experienced a 43% decrease in corticosterone from the DC phase (Figure 3); whereas, MAEM-SD experienced a 35% decrease. However, PC expressed a higher corticosterone level in RP than DC. This increase could be attributed to two rats (one in replicate 3 RP and the other in replicate 4 RP) which had abnormally high corticosterone levels compared to others in the same treatment. If these two data points were removed, a new mean for PC during RP of 27.17 ng/mL would be obtained, instead of 37.18 ng/mL as stated in Table 2. This new mean would be more consistent with 28.07 ng/mL which was the value obtained for PC during DC. It appears that MAEM-DD and MAEM-SD recovered during RP by expressing lower levels of corticosterone. However, there were no differences ($P > 0.05$) between treatments for both phases. Although there was a difference ($P < 0.05$) between replicates 3 and 4 for DC.

Total plasma proteins for DC and RP are presented in Table 3. During replicate 5 DC, the blood samples for each rat in MAEM-DD and MAEM-SD lysed; therefore, these samples were not assayed. This explains why MAEM-DD and MAEM-SD during DC have $n=4$ rather than $n=5$. PC had the highest level of plasma protein during DC while MAEM-SD had the highest during RP. Both MAEM-DD and PC showed a decrease at the end of RP while MAEM-SD showed an increase. Despite these differences, there were no differences ($P > 0.05$) between treatments during both phases. However, there were differences ($P < 0.05$) between replicates 1 and 4, 1 and 5, 2 and 4, 2 and 5, 3 and 4, and 3 and 5 for DC and replicates 1 and 5, 2 and 5, and 3 and 5 for RP.

Since there were no differences ($P > 0.05$) in plasma corticosterone levels between treatments, we expected no differences between neutrophil:lymphocyte (N:L) ratios. There were no differences ($P > 0.05$) between N:L between treatments, but there was a difference ($P < 0.05$) between replicates 1 and 5, 2 and 4, 2 and 5, and 3 and 5 within RP. This variation may have been due to slide preparation and error(s) in cell counting and not to treatment effects.

Leukocyte differential counts for DC and RP are presented in Table 4. The only difference ($P < 0.05$) between individual cell types was the percent of monocytes between MAEM-DD and PC during RP. Since there were so few monocytes present, this could be due to an error(s) in counting. There were differences ($P < 0.05$) among replicates within DC for eosinophils (1 and 2, 1 and 3, 1 and 4, and 1 and 5), band neutrophils (2 and 3, 2 and 4, and 2 and 5), lymphocytes (2 and 4), and monocytes (1 and 4, 1 and 5, 2 and 4, 2 and 5, 3 and 4, and 3 and 5). There were similar differences ($P < 0.05$) among replications within RP for eosinophils (1 and 2, 1 and 3, 1 and 4, 1 and 5, 2 and 3, and 2 and 5), segmented neutrophils (1 and 5, 2 and 4, 2 and 5, and 3 and 5), lymphocytes (1 and 5, 2 and 4, 2 and 5, and 3 and 5), and monocytes (1 and 4, 2 and 4, 3 and 4, and 4 and 5). Some of the rats which expressed high plasma corticosterone levels did exhibit an increase in neutrophil:lymphocyte ratio compared to rats with low plasma corticosterone. Calculating ratios by cage means masked these individual differences.

Packed cell volumes for DC and RP are presented in Table 5 and illustrated in Figure 5. MAEM-DD and MAEM-SD have higher cell volumes than PC. MAEM-DD and MAEM-SD at the end of RP had slightly lower volumes while PC remained the same. These treatment differences were not significant ($P>0.05$). However, a difference ($P<0.05$) did exist between replicates 1 and 5, 2 and 3, 3 and 5, and 4 and 5 within DC.

There are no adverse effects due to double density housing in MAEMs on male Sprague Dawley rats from the physiologic and immunologic parameters measured. There were differences among replicates but they did not cause treatment differences. Individual rats within the treatments did indicate the presence of stress by exhibiting increase plasma corticosterone levels and neutrophil:lymphocyte ratios. Only eight rats (out of 113 total) expressed high corticosterone levels exceeding 100 ng/mL (depending on the reference, levels over 100 ng/mL are still considered within baseline range). Four of these rats were from MAEM-DD, one from MAEM-SD, and three from PC. However, only 50% of those eight rats expressed a corresponding increase in neutrophil:lymphocyte ratios which would be expected. Therefore, the high corticosterone levels exhibited by the other 50%, may have been due to their response to the euthanasia procedure, instead of treatment, because the corticosterone did not have time to influence the leukocytes within the vasculature. Two of the eight rats also expressed a decrease in responsiveness to mitogen at all doses. Analyzing these parameters by cage means masked these individual effects. If a housing environment consistently produced stress, then a treatment stress effect should have been demonstrated. Since a treatment effect was not demonstrated in this experiment, the housing environments did not alter homeostatic processes to the point that stress symptoms were expressed.

Reference

Sas Institute, Inc. *SAS® User's Guide: Statistics, Version 5 Edition.* Cary, NC: SAS Institute Inc., 1985.

Table 1. Lectin-induced lymphocyte proliferation.

Treatment	Concanavalin A (ug/mL)	CPM[1]	
		DC[2]	RP
MAEM-DD[3]	24	39.3 ± 27.6*	38.3 ± 21.5
	12	296.1 ± 33.1	269.4 ± 58.2
	6	502.3 ± 22.9	463.5 ± 46.2
	3	457.9 ± 20.9	448.2 ± 28.7
	1.5	354.4 ± 25.9	382.4 ± 23.9
MAEM-SD	24	13.2 ± 6.7	43.6 ± 23.0
	12	255.6 ± 40.8	251.9 ± 52.3
	6	455.3 ± 47.2	494.8 ± 24.8
	3	427.7 ± 42.9	485.6 ± 18.9
	1.5	349.4 ± 31.9	407.9 ± 15.5
PC	24	8.5 ± 6.6	46.9 ± 28.5
	12	288.9 ± 53.1	269.7 ± 91.6
	6	488.6 ± 30.3	467.9 ± 32.3
	3	425.8 ± 35.4	457.9 ± 15.5
	1.5	321.4 ± 29.4	393.2 ± 9.8

*Values represent LSmeans ± SEM of CPM x 10^{-3}; n=5 for each treatment. Lectin-induced proliferation is reported as net responses with control (0 mitogen dose) subtracted.

[1]Determined by tritiated labeled thymidine incorporation by lymphocytes.

[2]DC represents 10 days of double density housing.
RP represents 10 days of normal density housing.

[3]MAEM-DD=Mock Animal Enclosure Module with 8 rats during DC and 4 rats in PC during RP.
MAEM-SD= Mock Animal Enclosure Module with 4 rats during DC and 4 rats in PC during RP.
PC=Standard Polycarbonate cage with 4 rats.

Table 2.[1] Plasma corticosterone in ng/mL

Treatment	DC	RP
MAEM-DD	45.21 ± 18.26*	25.55 ± 7.87
MAEM-SD	29.32 ± 10.34	18.97 ± 3.00
PC	28.07 ± 9.49	37.18 ±14.60

*Values represent LSmeans ± SEM where n=5 for each treatment.
[1]See Table 1 for abbreviation designations.

Table 3.[1] Total plasma protein in g/dL.

Treatment	DC	RP
MAEM-DD	6.29 ± 0.07*,†	6.25 ± 0.11
MAEM-SD	6.39 ±0 .15†	6.50 ± 0.17
PC	6.44 ± 0.10	6.27 ± 0.15

*Values represent LSmeans ± SEM where n=5 for each treatment.
†n=4.
[1]See Table 1 for abbreviation designations.

Table 4.[1] Leukocyte differential counts

Treatment		DC						RP					
	n	SN[a] %	L[b] %	M[c] %	EOS[d] %	BN[e] %	N:L ratio	SN %	L %	M %	EOS %	BN %	N:L ratio
MAEM-DD	5	8*	89	<2	<1	0	0.10±0.02**	10	88	<2†	<1	<1	0.11±0.03
MAEM-SD	5	10	88	<2	<1	<1	0.11±0.03	7	91	<2	<1	0	0.08±0.02
PC	5	9	89	<2	<1	<1	0.11±0.04	9	90	<1†	<1	<1	0.10±0.02

*Values represent means in percent.
**Values represent LSmeans ± SEM of N:L ratios.
†Significant at P<0.05 between MAEM-DD and PC.
[1]See Table 1 for abbreviation designations.
[a]Segmented neutrophil.
[b]Lymphocyte.
[c]Monocyte.
[d]Eosinophil.
[e]Band neutrophil.

Table 5.[1] % cell volume.

Treatment	DC	RP
MAEM-DD	44.50 ± 0.80*	44.30 ± 0.70
MAEM-SD	44.20 ± 0.50	45.10 ± 0.80
PC	43.80 ± 1.00	43.80 ± 0.40

*Values represent LSmeans ± SEM where n=5 for each treatment.
[1]See Table 1 for abbreviation designations.

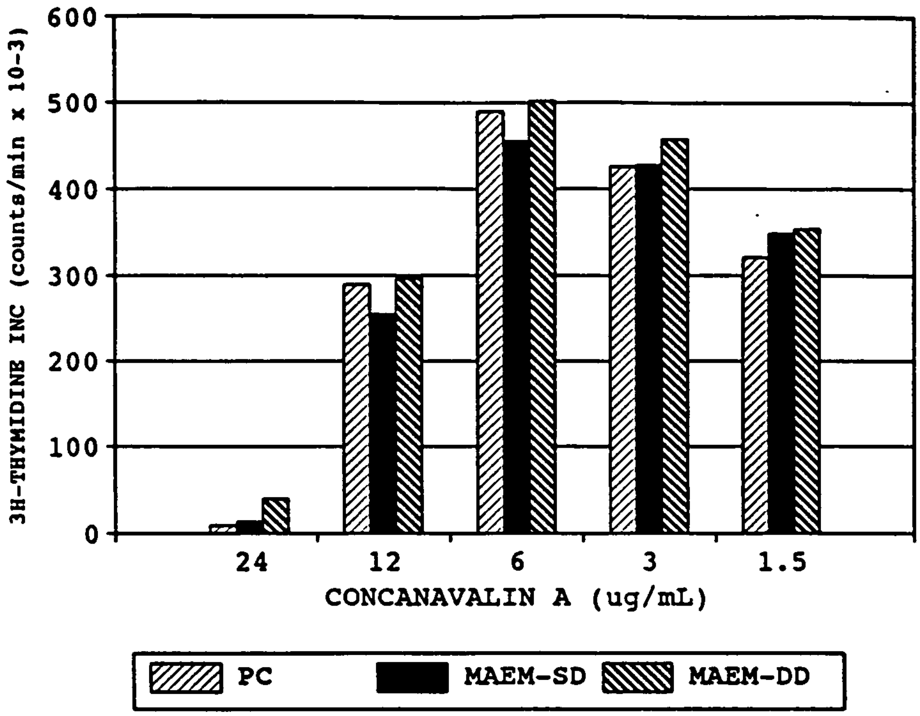

Figure 1. Lectin-induced lymphocyte proliferation, by treatment, for DC. SEMs are in Table 1.

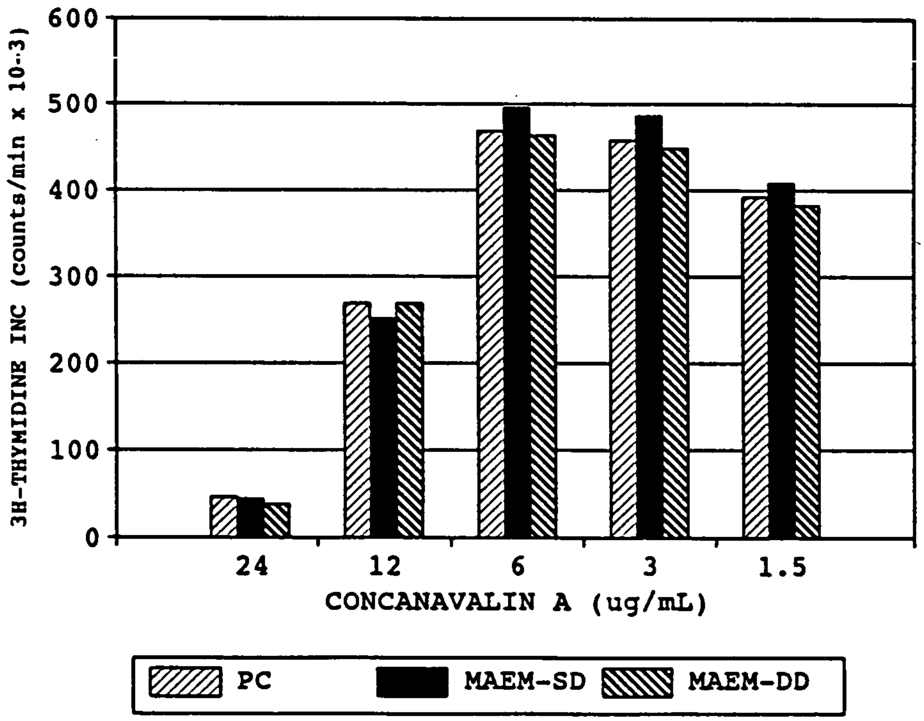

Figure 2. Lectin-induced lymphocyte proliferation, by treatment, for RP. SEMs are in Table 1.

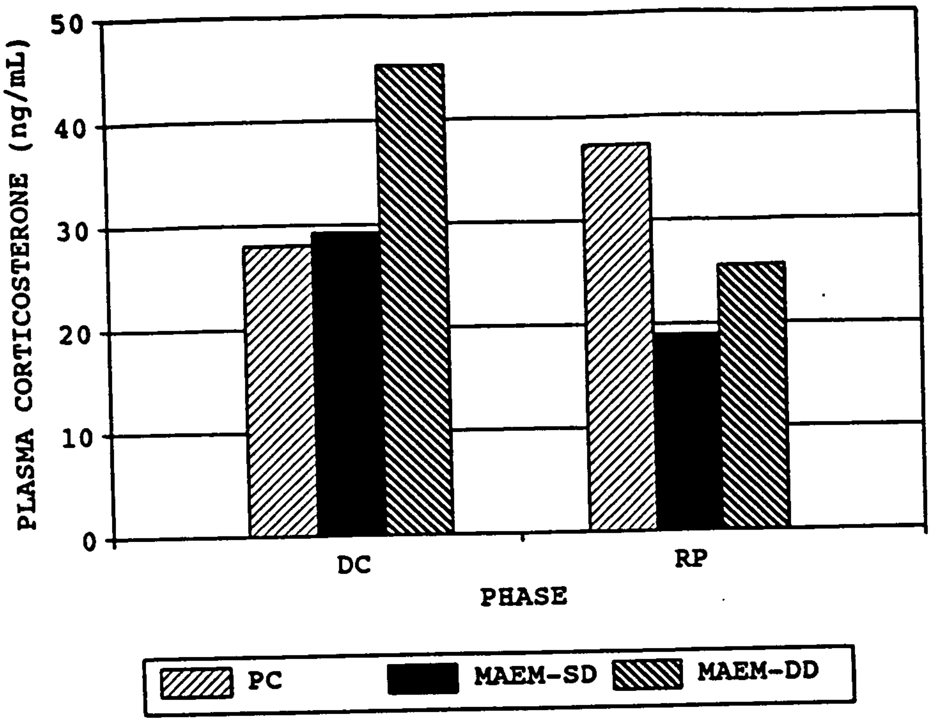

Figure 3. Plasma corticosterone, by treatment, for DC and RP.
SEMs are in Table 2.

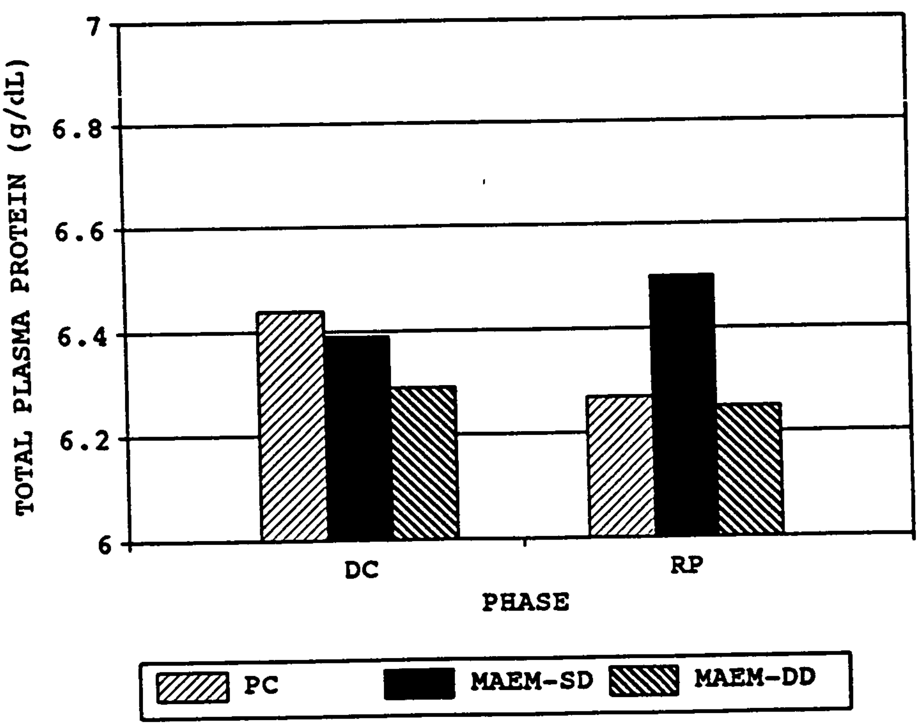

Figure 4. Total plasma protein, by treatment, for DC and RP.
SEMs are in Table 3.

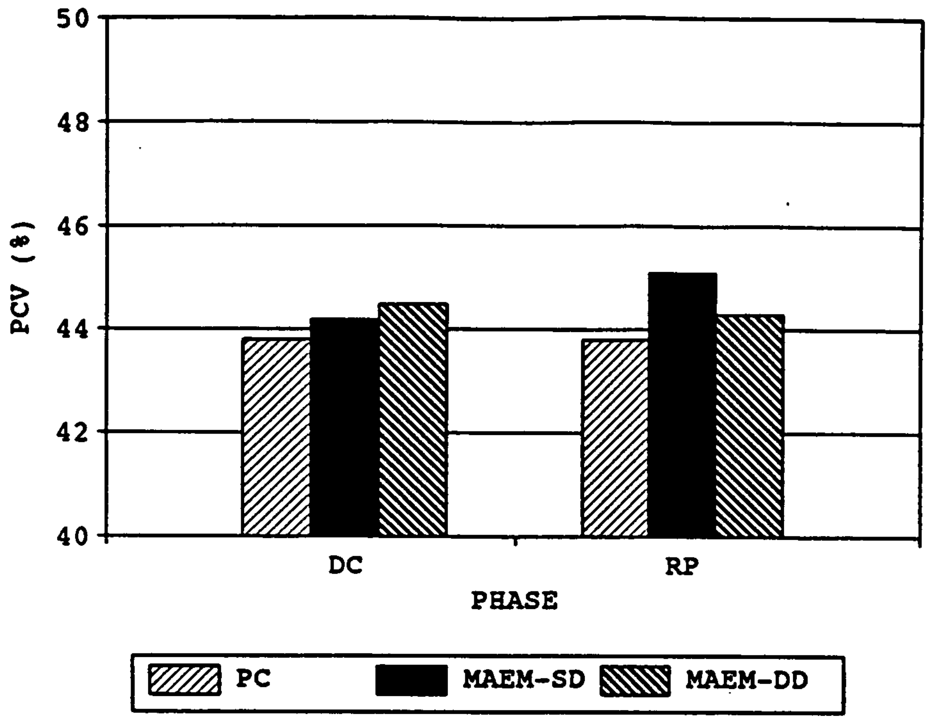

Figure 5. % cell volume, by treatment, for DC and RP.
SEMs are in Table 5.

Effect of Double Density Housing in MAEMs on Behavioral Activity Patterns of Male Sprague Dawley Rats

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Materials and Methods

During the DC phase of each replication, one cage from each treatment was videotaped for the first 48 hours and again on day 9 for 24 hours. For the PC and MAEM-SD cages one camera was positioned to take pictures from one end of the cage. Two cameras were used for the MAEM-DD (front and back) since it was difficult to monitor rat behavior at the higher density with just one camera. Black and white low light cameras with 0.5 lux minimum scene illumination were used. During the RP phase, all cages were videotaped for 24 hours on days 2 and 9. The camera signals were channeled through a switcher that changed the picture from cage to cage every 60 seconds during both phases.

The video tapes from days 2 and 9 of both phases were viewed to determine time budgets of: 1) standing, 2) standing erect on hind legs, 3) sitting, 4) lying, 5) licking, 6) eating, 7) drinking, 8) sleeping, 9) grooming, and 10) playing. The video tapes were scan sampled at one minute intervals for the first 20 minutes of each hour of the 24 hour period. Because the pictures were switched among 4 cages, each cage was scanned approximately five times in that 20 minute period. The huddling index was determined based on the method reported by Boon (1982). The huddling index was calculated four times each hour for each group and then pooled for each 4-hour period. The huddling index (HI) was calculated with the following equation:

$$HI = (\text{Number of rats lying in contact with others}) / (\text{Number of rats in group})$$

Rats were considered to be huddling when two or more rats were lying with some part of their body in contact with another rat's body. For example, with four rats to a cage the percentage of huddling could be 0%, 50%, 75%, or 100%. The area of floor not covered by rats was estimated by viewing the video screen to give the percent of free floor area.

Results and Discussion

Major activity levels (lying, sitting, standing normal, and standing erect) by cage type and phase of study are presented in Tables 1-2 and Figures 1-4. Eating, drinking, sleeping, grooming, and playing behavior did not show a pattern across the cage types and phases and are not reported. Split split-plot analyses were used to determine levels of significance for main effects (Table 3). Of the major activities, phase by cage-type interaction was significantly different for only lying frequency.

Rats in double density (MAEM-DD) cages during DC spent more ($P<0.05$) time lying (Figure 1). This greater lying time was reflected in numerically less standing in the normal position and less sitting, which approached significance ($P<0.10$). Erect standing did not show any trend across cage type nor phase. During the RP phase, the lying time of the MAEM-DD rats decreased to be essentially the same as the other treatments.

Figures 5-7 show similar patterns of lying behavior through the day for all treatments and phases; however, the MAEM-DD rats displayed more lying during the dark periods of the DC phase ($P<0.05$; Table 3). During DC, MAEM-DD rats showed a flatter diurnal pattern (especially at 1900 h) of lying activity than did PC and MAEM-SD rats in the DC. During RP, diurnal activity of the MAEM-DD rats returned to a diurnal pattern similar to rats in other groups.

During DC, the MAEM-DD rats had a significantly ($P<0.05$) higher huddling index than the PC or MAEM-SD rats (Figure 8). A higher huddling index would be expected in groups of rats experiencing cooler conditions, but probably was increased due to the closer proximity forced by the higher density of the MAEM-DD rats. There was significantly ($P<0.05$) less free floor area for MAEM-DD rats than the PC or MAEM-SD rats during the DC phase (Figure 9) as would be expected for higher density housing. However, the decrease in free floor area was not proportional to the percent of additional rats. A high free floor area indicates that the rats are staying close together, usually due to cold conditions. Since the free floor area decreased by more than double when the number of rats were doubled in the MAEM-DD cages, extra body heat may have kept them warmer. The fact that the MAEM-SD and PC rats had similar huddling indexes and free floor areas, would tend to indicate that the thermal comfort conditions were the same for both cage types at the same rat density.

Licking rate among rats can be affected by two or more factors. First, increased crowding could reduce convective heat loss which could lead to more acts of licking as a behavioral means of increasing evaporative heat loss. Second, crowding can lead to dirtier conditions in an enclosure which could lead to an increase in licking as a behavioral act associated with grooming or hair coat care.

Our data indicate that double density-caged rats did not engage in more licking, but instead engaged in less ($P < 0.05$) licking activity than rats housed less densely (Figure 10). The MAEM-DD rats engaged in 3.1 licks/minute/rat versus 4.6 and 4.8 for the MAEM-SD and PC rats, respectively. During RP, the rates of licking did not significantly differ among the three treatments. The physical appearance scores determined by Sebek indicated that MAEM-DD rats had less clean coats during DC than the lower density treatments, which would be consistent with lower licking rates.

There is a potential problem in comparing the rates of licking across the three cage types during DC. It was difficult to observe the licking behavior of all individual rats in the higher density cages. An additional camera was used at the back of the cage in an attempt to overcome this problem. However, there is still the possibility that some licking behavior by individuals in the center of the high density group occurred but was not observed. Licking is also possibly a displacement activity resulting from a thwarting of another behavioral drive. However, to determine the relevance of increased licking as a result of crowding, a more detailed investigation would be required.

Conclusion

Double density housing of rats resulted in between 5% and 10% more time spent lying. This decreased activity was reflected in less sitting and less normal standing. The amount of standing erect was not affected by doubling the density. The amount of licking decreased in double density housing. Other than decreased rates of activity, none of these behavioral differences was an obvious indicator of a state of negative well-being. All levels of activity returned to normal after the density was decreased.

References

Boon, C.R. 1982. The effect of air speed changes on the group postural behaviour of pigs. J. Agric. Engng. Res. 27:71-79.

Table 1. Standing, sitting, and lying activity levels (% of animals), licking (licks/rat/min), huddling index, and free floor area (% of floor area) for the DC phase.
Values are LSMeans±S.E.M.

ACTIVITY	TREATMENT		
	PC	MAEM-SD	MAEM-DD
Standing normal	5.92±0.79[a]	7.22±0.84[a]	3.45±0.44[b]
Standing erect	5.83±0.89[a]	7.40±0.95[a]	6.18±0.74[a]
Sitting	14.73±1.41[a]	14.86±1.17[a]	11.88±1.02[a]
Lying	73.51±2.52[a]	70.52±2.49[a]	78.49±1.65[b]
Licking Frequency	4.76±0.33[a]	4.58±0.36[a]	3.07±0.20[b]
Huddling index	0.14±0.02[a]	0.16±0.02[a]	0.24±0.01[b]
Free floor area	74.18±0.49[a]	74.36±0.70[a]	9.26±1.22[b]

[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$).

Table 2. Standing, sitting, and lying activity levels (% of animals), licking (licks/rat/min), huddling index, and free floor area (% of floor area) for the RP phase.
Values are LSMeans±S.E.M.
None of the means in the same row were significantly different ($P>0.05$).

ACTIVITY	TREATMENT		
	PC	MAEM-SD	MAEM-DD
Standing normal	6.93±0.71	7.81±0.65	7.99±0.58
Standing erect	6.39±0.80	6.60±0.92	6.89±0.87
Sitting	15.43±1.31	15.27±1.26	15.33±1.18
Lying	71.24±2.35	70.53±2.32	70.53±2.25
Licking Frequency	4.99±0.38	4.96±0.39	4.58±0.35
Huddling index	0.16±0.02	0.14±0.02	0.14±0.02
Free floor area	72.16±0.69	71.64±0.64	71.41±0.69

Table 3. Mean squares and levels of significance for effects as determined by Split Split-Plot Analysis.

Source	DF	Mean Squares			
		Lying	Sitting	Stand-norm	Stand-erect
Rep (R)	4	802.2**	273.0	65.9	115.1**
Treatment (T)	2	422.4*	93.8	74.4	47.0
R*T (Error 1)	8	60.2	82.4	47.3	13.4
Phase (P)	1	1124.3*	239.1*	373.3*	1.6
R*P	4	145.6	162.5*	29.3	31.0
T*P	2	502.4*	73.6	86.7	26.5
R*T*P(Error 2)	8	105.4	26.8	37.8	16.0
Day(Phase)	2	99.2	0.1	0.8	94.5
T*Day(Phase)	4	40.6	8.3	30.9	2.7
Hour(Day)	10	6281.8***	1578.6***	248.1***	635.5***
P*T*Hour	25	189.3**	50.1	28.0	20.4
Remainder	269	100.3	35.1	16.5	24.3

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

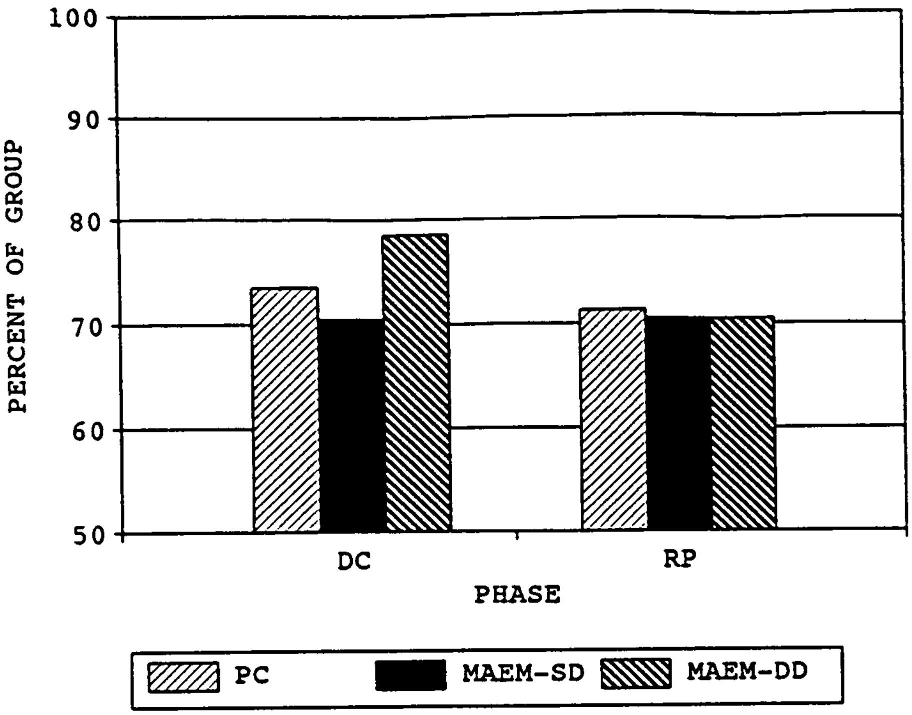

Figure 1. Lying activity by cage type and phase.
SEMs are given in Tables 1 and 2.

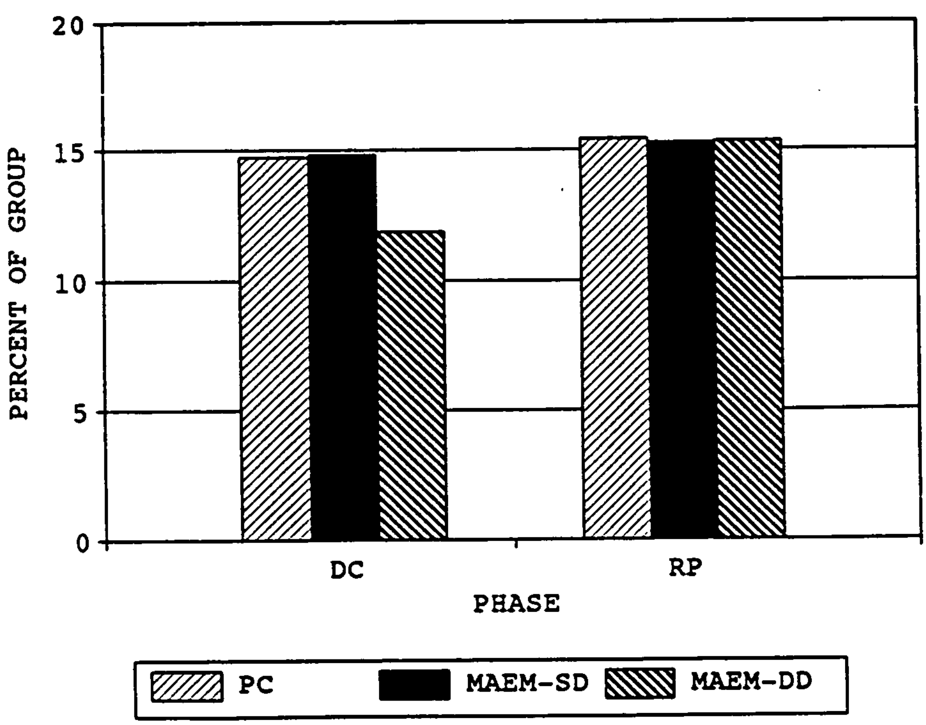

Figure 2. Sitting activity by cage type and phase.
SEMs are given in Tables 1 and 2.

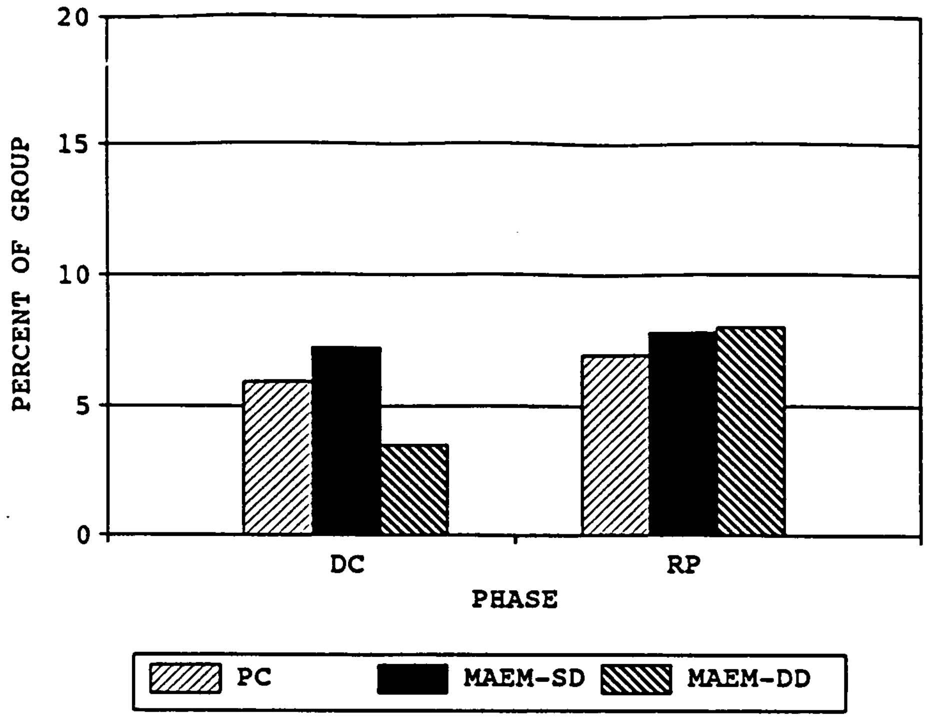

Figure 3. Normal standing activity by cage type and phase.
SEMs are given in Tables 1 and 2.

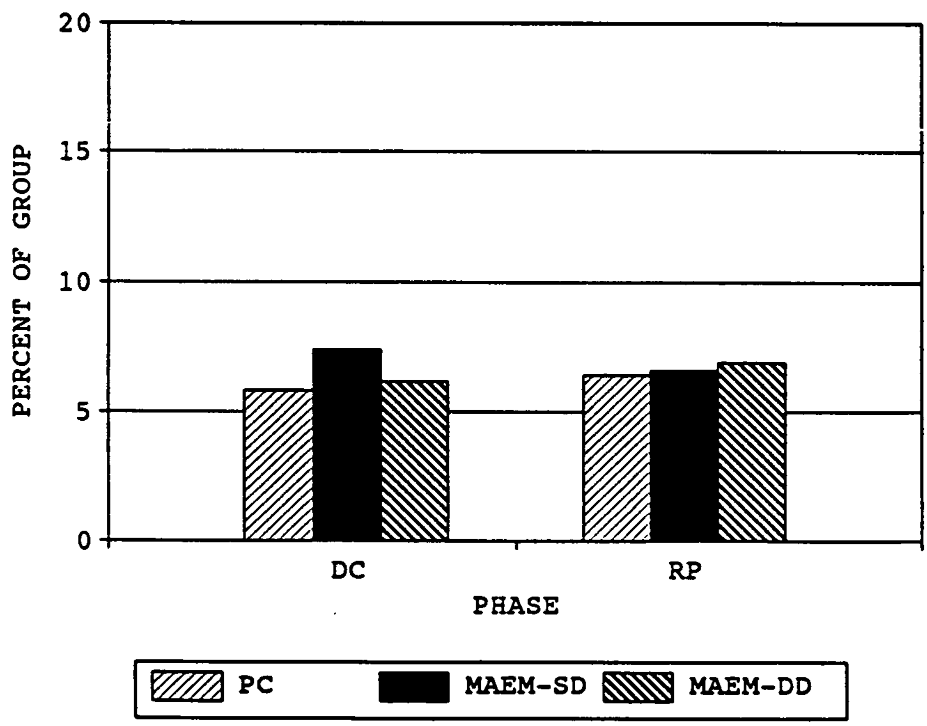

Figure 4. Erect standing activity by cage type and phase.
SEMs are given in Tables 1 and 2.

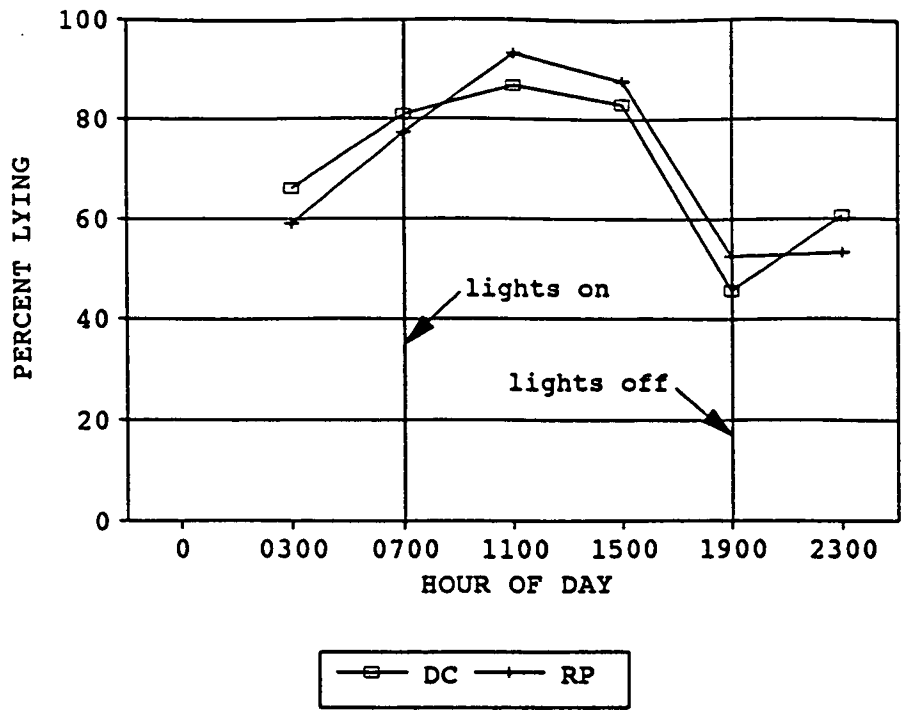

Figure 5. Diurnal lying activity for PC rats.

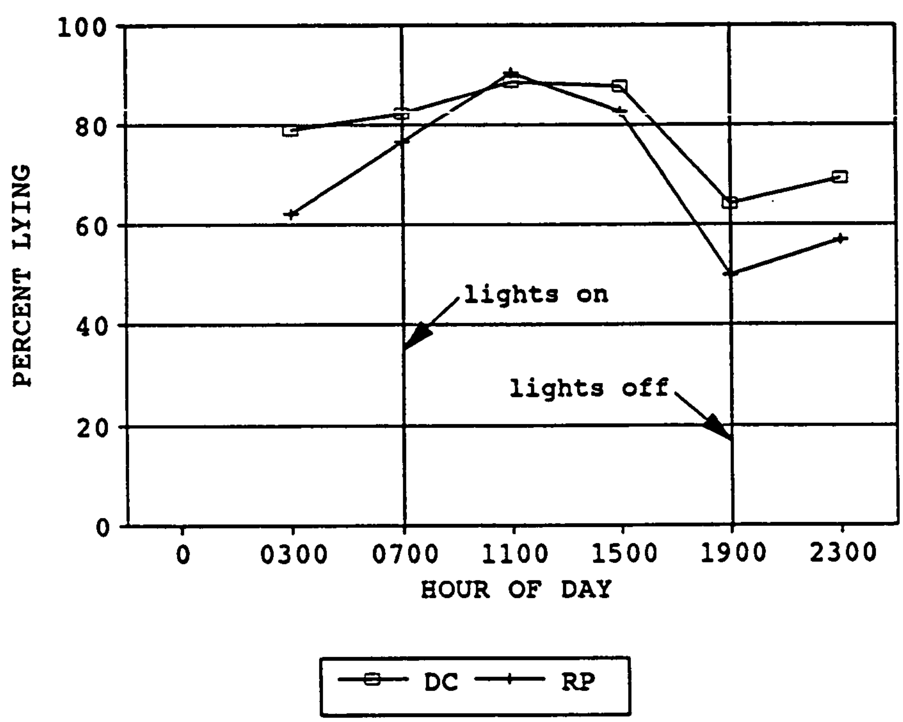

Figure 6. Diurnal lying activity for MAEM-DD rats.

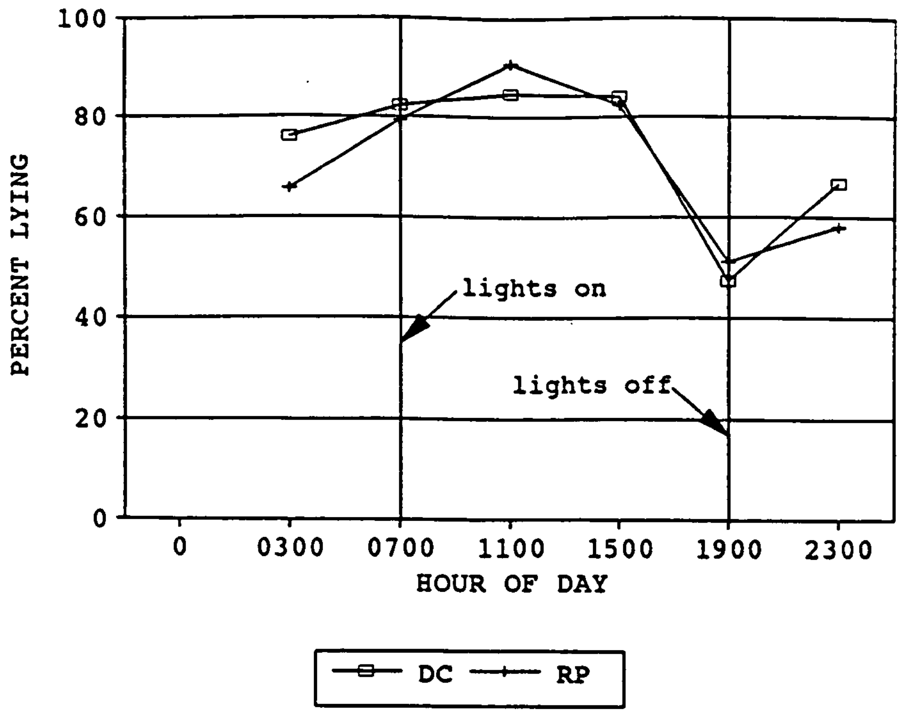

Figure 7. Diurnal lying activity for MAEM-SD rats.

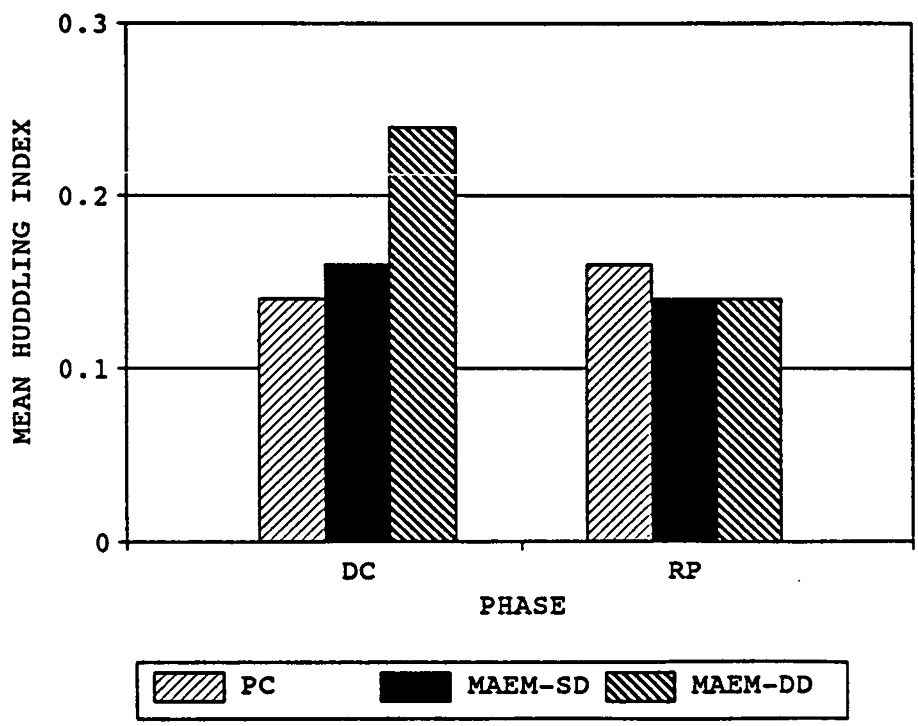

Figure 8. Mean huddling index by cage type and phase.
SEMs are given in Tables 1 and 2.

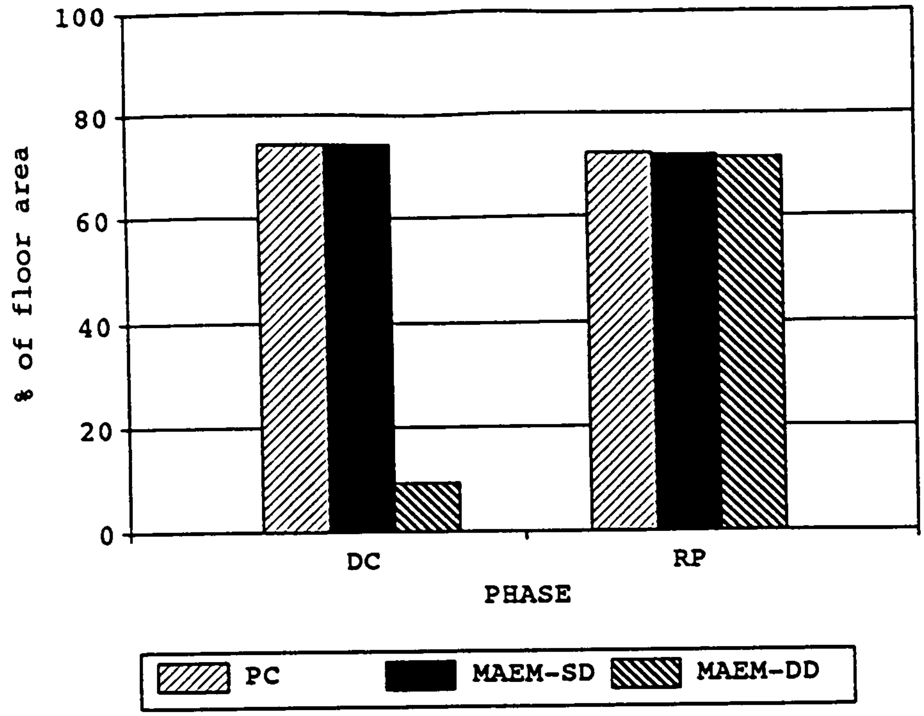

Figure 9. Free floor area by cage type and phase.
SEMs are given in Tables 1 and 2.

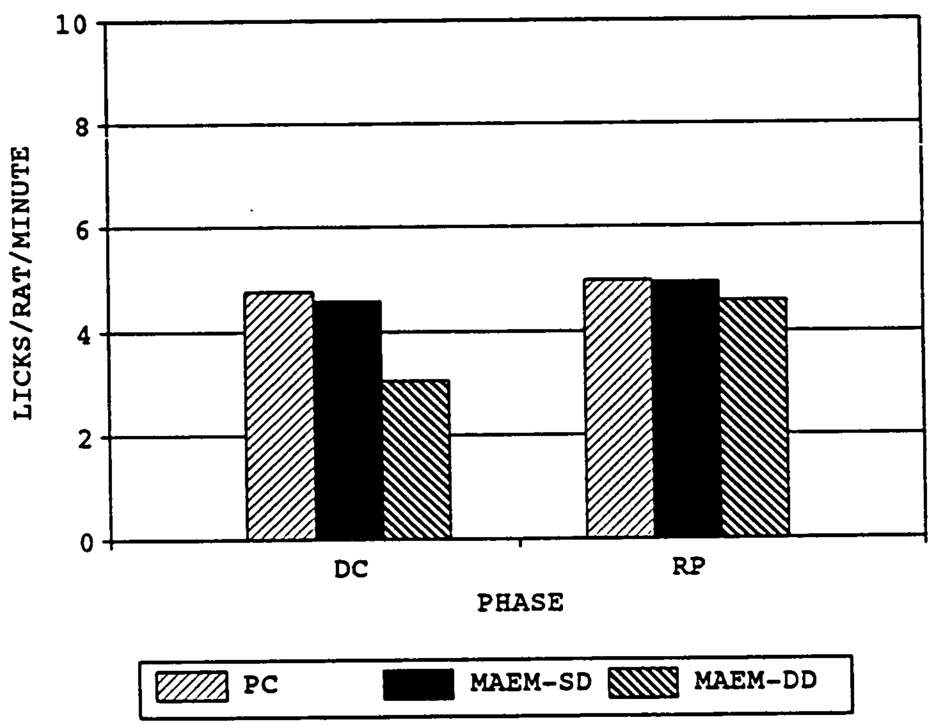

Figure 10. Licking frequency by cage type and phase.
SEMs are given in Tables 1 and 2.

Effect of Double Density Housing In MAEMs On Gross and Histopathology of the Gastrointestinal Tract (GIT) and Endocrine Systems

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Materials and Methods

For the gross tissue evaluation, the rats were necropsied and adrenals, thymus, and testes were removed, trimmed of excess tissue, weighed, and placed in formalin. The GI tract was tied at the esophageal opening to the stomach and the colon rectal area and removed. The GI tract and contents were weighed, flushed, weighed again (empty), and preserved in formalin. The tissue weights were calculated as a percentage of total body weight and means were calculated on a per cage basis (Table 5 of report by Sebek). Gross evaluations of GI mucosa from the stomach, duodenum, and ileum were made, with special notice for evidence of ulcerations and the occurrence of circumscribed distentions on the serosal surface.

For histologic evaluation of the GI tract and endocrine tissue, samples of gastric stomach, duodenum, ileum, large intestine, adrenals, testis, thymus, and the right femur were removed from each sacrificed rat and fixed in 10% buffered formalin solution. Bone tissue (femur) was decalcified in a solution of formic acid and sodium nitrate mixture before processing and staining. All tissues were processed at the Veterinary Histopathology Laboratory of the University of Illinois at Urbana-Champaign, and stained with standard hematoxylin-eosin. Tissue samples were examined microscopically for histologic evaluation.

Since some circumscribed distentions on the serosal surface and histopathologic effects were observed on the gastrointestinal tracts of experimental rats in replications 1 and 2, four non-trial rats that had been fed high-fiber chow diets were compared with experimental rats which were fed only NASA food bars. This small preliminary study was conducted to determine if there was any evidence that the NASA food bars may be causing the observed abnormalities. Two of the non-trial rats (rats 1 and 2) were first fed the NASA food bars for two weeks during an acclimation phase and then fed the high-fiber chow diet for 10 days (DC phase). The other two non-trial rats (rats 3 and 4) were fed only the high-fiber chow diet for the same period. All non-trial comparison rats were housed in PCs during both phases and were sacrificed for analysis after the DC phase. Comparisons were made on the gross pathologic and microscopic examinations of the gastrointestinal tract.

Results and Discussion

Tissue weights as a percent of body weight are presented in Table 5 of the report by Sebek. There were no differences ($P>0.05$) among the MAEM treatments and the PC treatment during either the DC or RP phase for any of the tissue weights, except for adrenals. MAEM-DD and MAEM-SD adrenals weights were higher ($P<0.005$) than PC during DC, but PC adrenals weights were higher ($P<0.05$) than the MAEM treatments during RP.

Gross evaluation of the GI mucosa from the stomach, duodenum, and ileum of all rats revealed no evidence of ulceration developed during the investigation across treatments. Gross pathologic examination revealed the occurrence of circumscribed distentions on the serosal surface, along the gastrointestinal tract, except on the surface of the duodenum, of all the rats, irregardless of treatment (Table 1). Mean distention counts were lower for rats in DC than RP.

Split-plot analysis with phase as the subplot and treatment as the mainplot was used to determine treatment and phase effects on mean number of distentions. Results showed no significant ($P>0.05$) difference among the treatments. Phase - treatment interactions and phase effects were also not significant ($P>0.05$).

During the DC phase, mean number of distentions were 7.4 ($\pm$SEM=1.2), 7.4(2.5), and 7.8(2.4) for the PC, MAEM-SD and MAEM-DD rats, respectively (Fig. 1). During the RP phase, mean number of distentions were 8.2(1.1), 8.8(1.2), and 10.4(1.2), for the PC, MAEM-SD, and MAEM-DD rats, respectively.

Rectal hemorrhages and hard pelleted stools were observed in some of the rats, independent of housing. These observations indicate an irritation in the GI tract which may be an inherent problem to the rats or problems with the food bars.

The four high-fiber chow fed rats had fewer distentions, and larger and longer gastrointestinal tracts (about twice the length) than the NASA food bar rats. Since distentions were observed in all rats, other inherent factors may be responsible. However, the four higher fiber chow rats did not give a controlled study, and further studies are needed to determine the effects of the food bar diet on the gastrointestinal tract of the rat.

Microscopic examination of the stomach revealed mild catarrhal inflammation of the glandular stomach for most rats (Table 2). The ileal histology showed mild infiltration of lymphocytic cells in most rats. Several rats had prominent Peyer's Patches and, in addition, some of these developed mild catarrh. In some cases the ileal surfaces revealed hyperplastic lymphocytic elements in the mucosa. Some of the Peyer's Patches in this area were confluent to a degree suggestive of lymphosarcoma, which in some cases nearly penetrated the serosal wall and contained areas of hyperplasia foci suggestive of the so-called starry sky "appearance". The Peyer's Patches were hyperplastic, and confluent and, in certain cases, they developed lymphoid aggregates which were essentially malignant, as evidenced by hyperchromasia, variation in cell size, and peripheral extension. In some cases, the patches were confluent and hyperplastic and nearly extended through the serosal wall of the ileum, changes suggestive of early neoplasia. The endocrine tissues, including the testis, thymus, and adrenals, were normal, as were the bone and bone marrow.

Table 1. Mean number of circumscribed distensions per rat on the serosal surface of GIT.

REP	PHASE	Treatment MAEM-DD	MAEM-SD	PC
1	DC	2	2	4
	RP	11	8	7
2	DC	2	1	5
	RP	6	5	9
3	DC	10	12	9
	RP	13	12	7
4	DC	13	9	9
	RP	10	8	6
5	DC	12	13	10
	RP	12	11	12

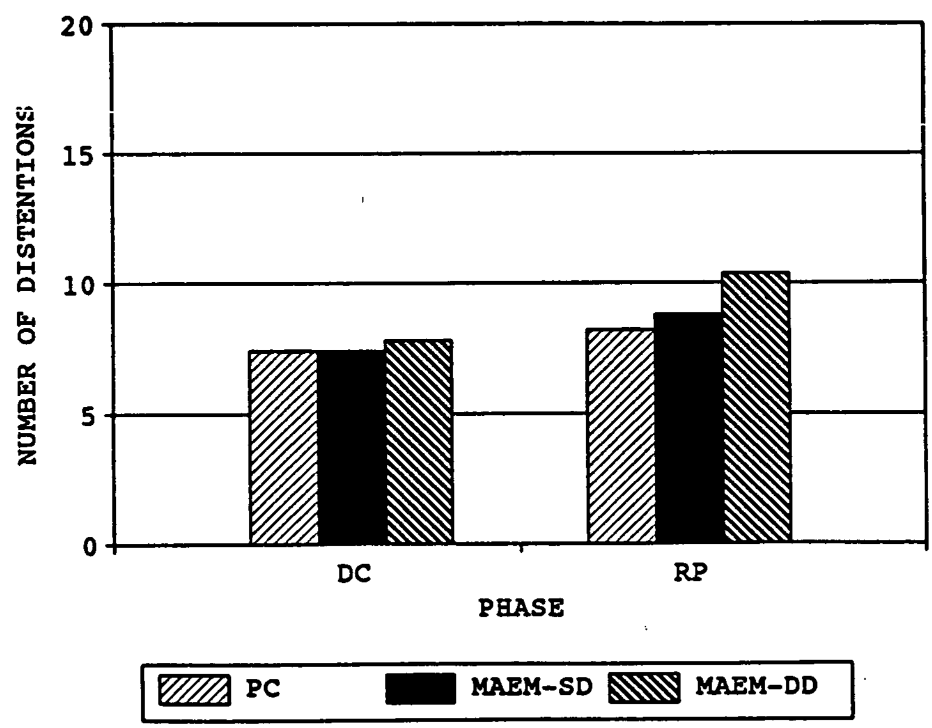

Figure 1. Mean number of distentions by cage type and phase.

Table 2. Results of microscopic examinations of GIT and endocrine elements and bone.

Replicate 1 - Density Challenge

Rat Identification #	Treatment	Stomach	Duodenum	Ileum	Adrenals	Testis	Thymus	Bones	Bones/Marrow
1DC	MAEM-DD	EN	M Infil	M Infil	EN	±	EN	EN	EN
2DC	MAEM-DD	MC	M Infil	M Infil	EN	±	EN	EN	EN
3DC	MAEM-DD	MC	M Infil	M Infil	EN	±	EN	EN	EN
4DC	MAEM-DD	EN	M Infil	M Infil	EN	±	EN	EN	EN
5DC	MAEM-SD	MC	M Infil	EN	EN	±	EN	EN	EN
6DC	MAEM-SD	MC	M Infil	PPP	EN	±	EN	EN	EN
7DC	MAEM-SD	MC	M Infil	M Infil	EN	±	EN	EN	EN
8DC	MAEM-SD	MC	M Infil	M Infil	EN	±	EN	EN	EN
9DC	PC	MC	M Infil	M Infil	EN	±	EN	EN	EN
10DC	PC	MC	M Infil	M Infil	EN	±	EN	EN	EN
11DC	PC	MC	M Infil	EN	EN	±	EN	EN	EN
12DC	PC	MC	M Infil	PPP	EN	±	EN	EN	EN

Replicate 1 - Recovery Phase

Rat Identification #	Treatment	Stomach	Duodenum	Ileum	Adrenals	Testis	Thymus	Bones	Bones/Marrow
1RP	MAEM-DD	EN	CPP	PPP	EN	±	EN	EN	EN
2RP	MAEM-DD	MC	CPP	PPP	EN	±	EN	EN	EN
3RP	MAEM-DD	MC	CPP	PPP	EN	±	EN	EN	EN
4RP	MAEM-DD	EN	CPP	PPP	EN	±	EN	EN	EN
5RP	MAEM-SD	MC	SSE	PPP	EN	±	EN	EN	EN
6RP	MAEM-SD	MC	CPP	PPP	EN	±	EN	EN	EN

Table 2. Continued

	7RP	MAEM-SD	MC	CPP	PPP	EN	±	EN	EN	EN
	8RP	MAEM-SD	MC	CPP	PPP	EN	±	EN	EN	EN
	9RP	PC	MC	CPP	PPP	EN	±	EN	EN	EN
	10RP	PC	MC	CPP	CAT-PP	EN	±	EN	EN	EN
	11RP	PC	MC	CPP	CAT-PP	EN	±	EN	EN	EN
	12RP	PC	MC	CPP	CAT-PP	EN	±	EN	EN	EN
Replicate 2 - Density Challenge										
	1DC	MAEM-SD	MC	MC	PPP	EN	±	EN	EN	EN
	2DC	MAEM-SD	MC	MMucInfil	PPP	EN	±	EN	EN	EN
	3DC	MAEM-SD	EN	EN	PPP	EN	±	EN	EN	EN
	4DC	MAEM-SD	EN	EN	PPP	EN	±	EN	EN	EN
	5DC	MAEM-DD	MC	EN	PPP	EN	±	EN	EN	EN
	6DC	MAEM-DD	MC	EN	PPP	EN	±	EN	EN	EN
	7DC	MAEM-DD	MC	EN	PPP	EN	±	EN	EN	EN
	8DC	MAEM-DD	EN	EN	PPP	MC	±	EN	EN	EN
	9DC	PC	MC	EN	PPP	EN	±	EN	EN	EN
	10DC	PC	MC	EN	PPP	MC	±	EN	EN	EN
	11DC	PC	MC	EN	PPP	EN	±	EN	EN	EN
	12DC	PC	MC	EN	CPP	EN	±	EN	EN	EN

Table 2. Continued

Replicate 2 - Recovery Phase									
1RP	PC	MC	EN	SSE	EN	±	EN	EN	EN
2RP	PC	MC	EN	SSE	EN	±	EN	EN	EN
3RP	PC	EN	EN	SSE	EN	±	EN	EN	EN
4RP	PC	EN	EN	SSE	EN	±	EN	EN	EN
5RP	MAEM-DD	MC	MMucInfil	SSE	EN	±	EN	EN	EN
6RP	MAEM-DD	EN	EN	SSE	EN	±	EN	EN	EN
7RP	MAEM-DD	MC	EN	SSE	EN	±	EN	EN	EN
8RP	MAEM-DD	MC	EN	SSE	EN	±	EN	EN	EN
9RP	MAEM-SD	MC	EN		EN	±	EN	EN	EN
10RP	MAEM-SD	MC	EN	SSE	EN	±	EN	EN	EN
11RP	MAEM-SD	MC	EN	SSE		±	EN	EN	EN
12RP	MAEM-SD	MC	EN	SSE	EN	±	EN	EN	EN
Replicate 3 - Density Challenge									
1 DC	MAEM-SD	EN	EN	SSE	EN	±	EN	EN	EN
2 DC	MAEM-SD	EN	EN	SSE	EN	±	EN	EN	EN
3 DC	MAEM-SD	EN	EN	SSE	EN	±	EN	EN	EN
4 DC	MAEM-SD	EN	EN	CPP	EN	±	EN	EN	EN
5 DC	PC	EN	EN	SSE	EN	±	EN	EN	EN
6 DC	PC	MC	EN	SSE/CPP	EN	±	EN	EN	EN
7 DC	PC	MC	EN	SSE	EN	±	EN	EN	EN
8 DC	PC	EN	EN	CAT-PP	EN	±	EN	EN	EN
9 DC	MAEM-DD	EN	M Infil	CPP	EN	±	EN	EN	EN

Table 2. Continued

	10 DC	MAEM-DD	EN	EN	CPP	EN	±	EN	EN	EN
	11 DC	MAEM-DD	EN	M Infil	CPP	EN	±	EN	EN	EN
	12 DC	MAEM-DD	EN	EN	CPP	EN	±	EN	EN	EN
Replicate 3 - Recovery Phase										
	1 RP	MAEM-SD	EN	EN	SSE	EN	EN	EN	EN	EN
	2 RP	MAEM-SD	MC	EN	SSE	EN	EN	EN	EN	EN
	3 RP	MAEM-SD	MC	EN	SSE	EN	EN	EN	EN	EN
	4 RP	MAEM-SD	EN	EN	SSE	EN	EN	EN	EN	EN
	5 RP	MAEM-DD	EN	EN	PPP	EN	EN	EN	EN	EN
	6 RP	MAEM-DD	MC	EN	PPP	EN	EN	EN	EN	EN
	7 RP	MAEM-DD	MC	EN	PPP	EN	EN	EN	EN	EN
	8 RP	MAEM-DD	MC	EN	PPP	EN	EN	EN	EN	EN
	9 RP	PC	MC	EN	PPP	EN	EN	EN	EN	EN
	10 RP	PC	MC	EN	PPP	EN	EN	EN	EN	EN
	11 RP	PC	MC	EN	PPP	EN	EN	EN	EN	EN
	12 RP	PC	MC	EN	PPP	EN	EN	EN	EN	EN
Replicate 4 - Density Challenge										
	1 DC	MAEM-DD	EN/MC	M Infil	CPP	EN	EN	EN	EN	EN
	2 DC	MAEM-DD	EN/MC	M Infil	EN	EN	EN	EN	EN	EN
	3 DC	MAEM-DD	EN/MC	M Infil	EN	EN	FDST	EN	EN	EN
	4 DC	MAEM-DD	EN/MC	M Infil	CPP	EN	EN	EN	EN	EN
	5 DC	MAEM-SD	EN	M Infil	CPP	EN	EN	EN	EN	EN
	6 DC	MAEM-SD	EN	M Infil	EN	EN	FDST	EN	EN	EN

Table 2. Continued

	7 DC	MAEM-SD	EN	M Infil	EN	EN	EN	EN	EN	EN
	8 DC	MAEM-SD	Ly Infil	M Infil	HPP	EN	EN	EN	EN	EN
	9 DC	PC	EN/MC	M Infil	PPP	EN	EN	EN	EN	EN
	10 DC	PC	EN/MC	M Infil	RPP	EN	EN	EN	EN	EN
	11 DC	PC	EN/MC	M Infil	RPP	EN	EN	EN	EN	EN
	12 DC	PC	EN/MC	M Infil	SSE	EN	EN	EN	EN	EN
Replicate 4 - Recovery Phase										
	1 RP	PC	EN/MC	LyInfil V	RPP	EN	EN	EN	EN	EN
	2 RP	PC	EN	LyInfil V	EN	EN	EN	EN	EN	EN
	3 RP	PC	LyInfil V	LyInfil V	EN	EN	EN	EN	EN	EN
	4 RP	PC	EN	LyInfil V	RPP	EN	EN	EN	EN	EN
	5 RP	MAEM-SD	EN	LyInfil V	RPP	EN	EN	EN	EN	EN
	6 RP	MAEM-SD	EN	LyInfil V	EN	EN	EN	EN	EN	EN
	7 RP	MAEM-SD	EN/MC	Ly Infil	EN	EN	EN	EN	EN	EN
	8 RP	MAEM0SD	EN/MC	SSE	EN	EN	EN	EN	EN	EN
	9 RP	MAEM-DD	EN	MC	RPP	EN	EN	EN	EN	EN
	10 RP	MAEM-DD	EN/MC	M Infil	EN	EN	EN	EN	EN	EN
	11 RP	MAEM-DD	EN	M Infil	RPP	EN	EN	EN	EN	EN
	12 RP	MAEM-DD	EN	M Infil	SSE	EN	EN	EN	EN	EN
Replicate 5 - Density Challenge										
	1 DC	MAEM-DD	MC/EN	M Infil	PPP	EN	EN	EN	EN	EN
	2 DC	MAEM-DD	MC/EN	M Infil	RPP	EN	EN	EN	EN	EN
	3 DC	MAEM-DD	MC/EN	M Infil	HPP	EN	EN	EN	EN	EN

Table 2. Continued

4 DC	MAEM-DD	-	MMucInfil	HPP	EN	EN	EN	EN	EN
5 DC	MAEM-SD	MC/EN	MMucInfil	HPP	EN	EN	EN	EN	EN
6 DC	MAEM-SD	MC/EN	MMucInfil	HPP	EN	EN	EN	EN	EN
7 DC	MAEM-SD	MC/EN	MMucInfil	HPP	EN	EN	EN	EN	EN
8 DC	MAEM-SD	MC/EN	MMucInfil	HPP	EN	EN	EN	EN	EN
9 DC	PC	MC/EN	MMucInfil	CPP	EN	EN	EN	EN	EN
10 DC	PC	MC/EN	MMucInfil	RPP	EN	EN	EN	EN	EN
11 DC	PC	MC/EN	MMucInfil	SSE	EN	EN	EN	EN	EN
12 DC	PC	MC/EN	MMucInfil	SSE	EN	EN	EN	EN	EN
Replicate 5 - Recovery Phase									
1 RP	MAEM-SD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
2 RP	MAEM-SD	EN/MC	LyInfil V	EN	EN	EN	EN	EN	EN
3 RP	MAEM-SD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
4 RP	MAEM-SD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
5 RP	PC	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
6 RP	PC	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
7 RP	PC	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
8 RP	PC	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
9 RP	MAEM-DD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
10 RP	MAEM-DD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
11 RP	MAEM-DD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN
12 RP	MAEM-DD	EN/MC	LyInfil V	CPP	EN	EN	EN	EN	EN

Table 2. Continued

Controls									
1	EN/MC	MMucInfil	RPP	--	--	--	--	--	--
2	EN/MC	MMucInfil	RPP	--	--	--	--	--	--
3	EN/MC	MMucInfil	RPP	--	--	--	--	--	--
4	EN/MC	MMucInfil	RPP	--	--	--	--	--	--

Key to notations:

DC = Density Challenge
RP = Recovery Phase
DD = Double Density in MAEM
SD = Single Density in MAEM
PC - Polycarbonate in cage
EN = Essentially Normal
MC = Mild Catarrh
MMucInfil = Mild mucosal infiltration
PPP = Prominent Peyer's Patches
M Infil = Mild Infiltration
CAT-PP = Catarrhal Peyer's Patches
SSE = Starry Sky Effect
CPP = Confluent Peyer's Patches
FDST = Few degenerating seminiferous tubules
HPP = Hyperplastic Peyer's Patches
RPP = Reactive Peyer's Patches
Ly Infil = Lymphoid Infiltration
LyInfil V = Lymphatic infiltration of villi
± = More or less normal.
EN/MC = Essentially with Milk Catarrh.