Effect of Cage Density and Sex on Growth, Food Utilization and Some Stress Parameters of Young Rabbits

by ***E. Ebru Onbaşılar[1,*] & İlyas Onbaşılar[2]***

[1,*]Ankara University, Faculty of Veterinary Medicine, Department of Animal Science, Dışkapı, Ankara, Turkey

[2]Hacettepe University, Faculty of Medicine, Laboratory Animal Husbandry and Research Unit, Ankara, Turkey

Summary

The aim of the experiment was to study the effects of cage density (1, 3 and 5 rabbits per cage) and sex (male and female) on stress parameters of young rabbits. A total of 90 (45 male and 45 female) weaned New Zealand White rabbits aged 35 days old were used in the experiment. Rabbits were allocated as 1, 3 and 5 rabbits, in each of 5 cages, to obtain three different cage density groups: 4200, 1400 and 840 cm^2 floor area per rabbit, respectively. Mean values for total body weight gain, food intake, food:gain ratio, the plasma corticosterone level and serum levels of glucose, cholesterol and triglycerides were taken as indicators of stress. The group having 5 rabbits per cage had significantly lower total body weight gain ($p<0.001$), food intake ($p<0,001$) and higher food:gain ratio ($p<0,01$) than other groups during the experiment. The levels of plasma corticosterone and serum glucose were higher ($p<0.001$) in the group with 5 rabbits per cage than other groups. Values for serum levels of cholesterol and triglyceride were not affected by cage density. Gender effect was detected only in corticosterone level. Male rabbits had higher plasma corticosterone than female rabbits. The results suggest that the allocation of 1 or 3 rabbits per cage had no measurable adverse effects on the welfare of male and female young rabbits, whereas (at our cage densities) 5 ones.

Introduction

Laboratory animals such as rabbits are still used widely in many experiments to contribute the knowledge in scientific research. Generally they are housed in cages for single animals or groups. The optimal group size is determined by sex and age of the animals, cage size and experimental design. For rabbits, minimum space allowances and stocking densities should always refer to the final weight that rabbits would reach (of a certain strain, sex, feeding regime) while housed in a particular compartment. In cage housing, each rabbit should be allowed to stretch full length along one side of the cage. The height should allow the rabbits to sit up straight. Young rabbits need more space since they are more active and perform more rapid locomotion than elders (*Lehmann, 1987*).

Recommended cage area for rabbits is different in the US and European guidelines. In the NRC (1996), cage floor area is based on weight, such that one rabbit of less than 2 kg should be housed in a cage with a minimum floor area of 1350 cm^2. In the Council of Europe Appendix A ETS 123 (*CoE, Strasbourg, 2004*), cage floor area is based on weight and age, such that rabbits less than 10 weeks of age should be housed in a cage with a minimum floor area of 1200 cm^2.

For young rabbits, it is beneficial to stay together with litter-mates, as long as possible. The laboratory animals must have freedom to express as much

**Correspondence:* E. Ebru Onbaşılar
Ankara University, Faculty of Veterinary Medicine,
Department of Animal Science, 06110 Dışkapı, Ankara, Turkey
Tel: +90 312 3170315 / or 311
Fax: +90 312 3181758
E-mail: obasilar@veterinary.ankara.edu.tr

natural behavior as possible including play and social contact if appropriate. The inability to exhibit a full range of natural behaviors is detrimental to health and welfare causing poor physiological well-being. This leads to stress, which can cause errors in the experimental results (*Wolfensohn* & *Lloyd, 2003; Chu et al, 2004*).

Podberscek et al. (*1991*) found that the space restrictions of standard cages (2600 cm^2) frustrate normal hopping locomotion and that isolated rabbits spent more time on self-directed grooming, were more restless and exhibited more stereotypic behavior compared with group-housed rabbits. Also Love and Hammond (*1991*) observed that space restriction and the isolation of single housing prevented mutual grooming, play and the erect 'prairie dog' posture that rabbits assume when investigating disturbances. Turner et al. (*1997*) showed that group-housed does are suitable for raising antisera.

The effects of cage density on performance and behavior had been studied by different authors (*Love and Hammond, 1991; Morisse and Maurice, 1997; Trocino et al., 2004*). However, as far as we know, there are no published reports about the effects of stress due to different cage density on growth and some blood parameters in male and female young rabbits. Therefore, the aim of this study was to investigate the effects of cage density and sex on total body weight gain, food utilization and some blood parameters of young rabbits.

Materials and Methods

Animals and diet

A total of 90 (45 male and 45 female) weaned New Zealand White rabbits aged 35 days old were used in this study. Initial body weight (mean±SEM) was 766±15 g for males and 787±11 g for females. They were obtained from Hacettepe University Laboratory Animal Husbandry and Research Unit and housed in stainless steel cages (70 cm x 40 cm x 60 cm, width x height x depth respectively) with perforated floors. Bedding material was not used. The microbiological status of the rabbits was not specified. The animals were maintained under standard laboratory conditions (temperature from 16 to 20^{0}C, relative humidity from 48 to 68% and 12 h light/dark cycles). They had free access to a standard pellet diet (6mm in diameter and 2 cm in length) obtained from a specialist manufacturer (Korkutelim, Antalya, Turkey). The diet had 180 g crude protein, 175 g acid detergent fiber and 12.57 MJ digestible energy per kg. Tap water was available *ad libitum*. All experimental manipulations were conducted during the light phase. All animal-use protocols were in accordance with the regulations outlined by the Hacettepe University Committee on Laboratory Animals.

Experimental Design

Both sex groups were divided into three groups having different cage densities (1, 3 and 5 male or female rabbits/cage). Groups didn't contain litter-mates. Each group consisted of 5 replicates. The floor area per rabbit was 4200, 1400 and 840 cm^2 in the groups having 1, 3 and 5 rabbits/cage, respectively. The experiment lasted 6 weeks.

Traits Measured

Rabbits were weighed individually every week. Food consumption was determined precisely each week and calculated as g per rabbit per week. Unused food from each cage was collected daily, weighed and taken into consideration for calculation of food consumption. The food:gain ratio was calculated as kg food per kg body weight gain. Blood samples were collected by cardiac puncture at the end of the experiment. Blood samples were taken from each of the rabbits in cages having one rabbit, and from a chosen, socially "subordinate" rabbit in cages having 3 and 5 rabbits. The bleeding was performed under general anesthesia, which was induced by intramuscular injection of a mixture of 40 mg/kg body weight ketamine hydrochloride (Ketalar, Eczacıbası, Istanbul, Turkey) and 10 mg/kg xylazine (Alfasan, Woerden, Holland). Blood samples were taken in two tubes, one contained EDTA for measuring plasma corticosterone levels and the other had no anticoagulant for measuring serum cholesterol, glucose and triglyceride levels. The bleeding procedure was limited to 1 min or less to minimize the influence of handling stress. All blood samples were collected at the same time in the morning and centrifuged.

Plasma samples were frozen (-20°C) until analyzed for corticosterone determination. Plasma corticosterone levels were measured using the kits (Gamma-B ^{125}I Corticosterone, Code AA-13 F1) for IDS double antibody RIA technique, with a Berthold LB211 gamma counter.

Serum concentrations of cholesterol, glucose and triglyceride were determined by Hitachi autoanalyser (Hitachi Ltd, Tokyo, Japan) using their accompanying commercial kits.

Statistical Analyses

Statistical analyses were performed using the SPSS software package for Windows (SPSS, Chicago, IL, USA). The experimental unit was the cage. Thus, the statistical model had 30 observations or cages in total. Data were tested for distribution normality and homogeneity of variance. One-way ANOVA was used to compare differences among groups in body weight gain and food:gain ratio. When a significant difference was found among cage groups, Duncan's test was used. A two-way ANOVA was used to determine the differences between cage density and sex and their interactions with respect to the studied parameters (*Dawson* & *Trapp, 2001*).

Results

There were no statistically significant differences among groups in initial body weight. The mean estimated values were 1147, 1191 and 989 g for total body weight gain, 3680, 3765 and 3491 g for total food intake during six weeks and 3.21, 3.17 and 3.54 for food:gain ratio during the experiment in groups having 1, 3 and 5 rabbits/cage, respectively.

Rabbits housed in cages having 5 rabbits had less total body weight gain ($p<0,001$) and higher food: gain ratio ($p<0.01$) than in cages with 1 or 3 rabbits (Table 1). When analysed weekly, these effects were detected from week 2 to week 6 ($p<0.05$, Figure 1). There was no cage density by sex interaction effect on the total body weight gain, food intake, or food: gain ratio. No gender effect was detected on total body weight gain, food intake, and food:gain ratio (Table 1).

Plasma corticosterone and serum glucose levels were significantly affected ($p<0,001$) from cage density as shown in Table 2. In contrast, serum cholesterol and triglyceride levels were similar

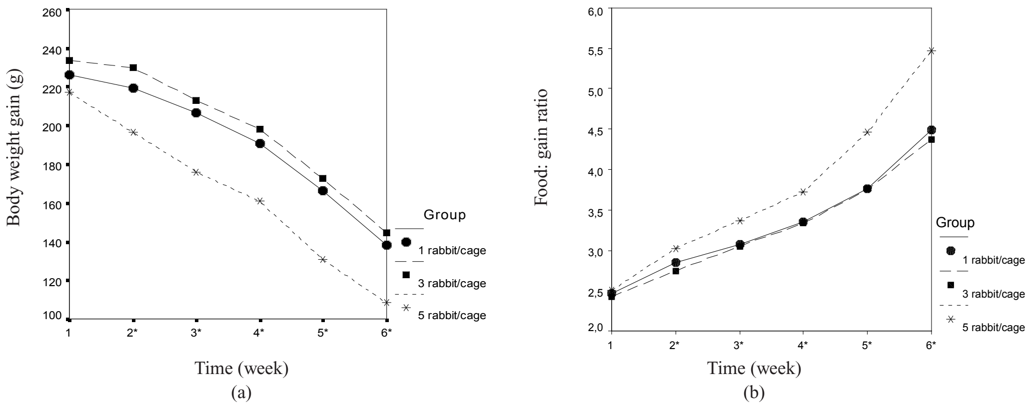

Figure 1. The effects of cage density on body weight gain (a) and food:gain ratio (b) during follow-up period.

* $p<0.05$, rabbits setting in cages having 5 rabbits had less total body weight gain and higher food:gain ratio than that having one or three rabbits from week 2 to week 6.

Table 1. Effects of cage density and sex on total body weight gain, total food intake and food:gain ratio.

Cage density	Sex	Total body weight gain (g)	Total food intake (g)	Food:gain ratio
1 rabbit/cage				
	M	1159±26	3632±49	3.13±0.09
	F	1134±26	3728±49	3.29±0.09
3 rabbits/cage				
	M	1187±26	3755±49	3.17±0.09
	F	1195±26	3776±49	3.17±0.09
5 rabbits/cage				
	M	976±26	3457±49	3.56±0.09
	F	1002±26	3525±49	3.52±0.09
Total				
	M	1108±15	3614±29	3.29±0.05
	F	1110±15	3676±29	3.328±0.05
1 rabbit/cage	MF	1147±18a	3680±35a	3.21±0.06a
3 rabbits/cage	MF	1191±18a	3765±35a	3.17±0.06a
5 rabbits/cage	MF	989±18b	3491±35b	3.54±0.06b

Two Way ANOVA (F)

MF	0.016	2.345	0.269
CD	33.856***	16.119***	10.041**
CDXMF	0.508	0.297	0.634

Values are estimated marginal mean ± SEM.
abc. xy: means results within columns with different letters are significantly different (p<0.05).
**p<0.01, ***p<0.001.
CD: cage density, MF male-female, MFXCD: MF by CD interaction.

Table 2. Effects of cage density and sex on plasma corticosterone level, serum glucose, cholesterol and triglyceride levels.

Cage density	Sex	Serum cholesterol (mmol/L)	Serum glucose (mmol/L)	Serum triglyceride (mmol/L)	Plasma corticosterone (nmol/L)
1 rabbit/cage					
	M	0.97±0.04	6.01±0.15	1.31±0.06	4.31±0.18
	F	0.93±0.04	5.95±0.15	1.39±0.06	4.21±0.18
3 rabbits/cage					
	M	0.92±0.04	5.65±0.15	1.32±0.06	4.66±0.18
	F	0.88±0.04	5.53±0.15	1.40±0.06	3.76±0.18
5 rabbits/cage					
	M	0.86±0.04	7.60±0.15	1.43±0.06	6.71±0.18
	F	0.84±0.04	7.47±0.15	1.27±0.06	6.44±0.18
Total					
	M	0.92±0.03	6.42±0.09	1.35±0.03	5.23±0.11x
	F	0.88±0.03	6.32±0.09	1.36±0.03	4.80±0.11y
1 rabbit/cage	MF	0.95±0.03	5.98±0.11b	1.35±0.04	4.26±0.13a
3 rabbits/cage	MF	0.90±0.03	5.59±0.11a	1.36±0.04	4.21±0.13a
5 rabbits/cage	MF	0.85±0.03	7.54±0.11c	1.35±0.04	6.57±0.13b
			Two Way ANOVA (F)		
MF		0.869	0.699	0.002	7.871*
CD		2.746	93.445***	0.006	107.318***
CDXMF		0.017	0.038	2.926	2.580

Values are estimated marginal mean ± SEM.
abc. xy: means results within columns with different letters are significantly different (p<0.05).
*p<0.05, ***p<0.001.
CD: cage density, MF male-female, MFXCD: MF by CD interaction.

among groups having 1, 3 and 5 rabbits/cage. Sex didn't affect the serum levels of cholesterol, glucose and triglyceride. However, male rabbits had higher plasma corticosterone than females ($p<0.05$). There was no cage density by sex interaction effect on the levels of plasma corticosterone, serum cholesterol, serum glucose and serum triglyceride.

Discussion

Groups having 1 and 3 rabbits/cage had higher mean values for total body weight gain and food intake than the group having 5 rabbits/cage. Therefore, the reduction of total body weight gain in the group with 5 rabbits/cage may be explained by lower food intake and lower physical activity due to the crowding stress, as observed by Morisse and Maurice (*1997*). Whary et al. (*1993*) reported that food intake was significantly higher in group-housed rabbits (but at only ¼ of our density) than single-housed rabbits. In the present study, groups having 5 rabbits/cage showed higher mean food:gain ratio than those held in cages with lower density. This may be due to the higher density that decreased the possibilities for movement. Similarly, Trocino et al. (*2004*) reported that food efficiency was better ($p=0.05$) in the groups having 625 cm^2 floor area per rabbit than in groups having 825 cm^2 floor area per rabbit. In our study body weight gain from week 2 to week 6 was significantly lower with 5 rabbits/cage than with 1 or 3 rabbits/cage; and food utilization was found to be better in groups with 1 or 3 rabbits/cage. There were no differences in body weight gain and food:gain ratio between 1 rabbit/cage and 3 rabbits/cage.

The Guide for the Care and Use of Laboratory Animals (*NRC, 1996*) recommended that a rabbit less than 2 kg should be housed in a cage with a minimum floor area of 1350 cm^2 and the Council of Europe, ETS 123 (*CoE, Strasbourg, 2004*) recommended that a rabbit less than 10 weeks of age should be housed in a cage with a minimum floor area of 1200 cm^2. Similarly in this study, rabbits less than 2 kg and less than 11 weeks of age showed a better growth and food:gain ratio in 1400 cm^2 than 840 cm^2 of floor area.

Change in plasma corticosterone concentration is considered to be a valid indicator for physiological stress. Plasma corticosterone levels of the groups having 1 rabbit/cage and 3 rabbits/cage were similar. Also Whary *et al.* (*1993*) reported that single and group housed female rabbits did not significantly differ in corticosterone level. In this study plasma corticosterone level of the group having 5 rabbits/cage was higher ($p<0.001$) than those of groups having 1 or 3 rabbits/cage, indicating that the rabbits in cages with 5 animals had more stress than the rabbits in cages having 1 or 3 animals. We also found that male rabbits produced higher plasma corticosterone levels than females. A related study showed a significant positive correlation between serum corticosterone level and group size in mice (*Barnard et al 1994*).

In this study, serum glucose level was higher in groups having 5 rabbits/cage than in groups of 1 and 3 rabbits/cage. Increasing serum glucose levels due to the effect of corticosterone are described as an important indicator of stress in chickens (*Simon, 1984*). We found serum cholesterol and triglyceride levels in different density groups of male and female rabbits to be similar. Similarly, Perez *et al.* (*1997*) found that there were no statistically significant differences in total cholesterol values of isolated and grouped rats.

Lethal and serious injuries due to fighting were not observed during our experiment, which may be due to the rabbits having been kept together from weaning and not reaching sexual maturity during the experiment.

Both the Council of Europe (*CoE, 1986*) and the Guide for the Care and Use of Laboratory Animals (*NRC, 1996*) recognize that rabbits are social creatures and should be housed, when possible, in social groups to maximize species-specific behaviors and minimize stress. However, in this study, young rabbits caged individually or in groups of 3 rabbits/cage were not different in body weight gain, food:gain ratio and stress parameters such as plasma corticosterone level; however there was some negative impact at the rabbis/cage density.

In conclusion; a gender effect was not detected in the studied parameters except that in corticosterone levels. Increasing cage density from 1 and 3 to 5 rabbits/cage resulted a decrease in growth, food intake, an increase in food:gain ratio, plasma corticosterone level and serum glucose level. Therefore it appears that the allocation of 1 or 3 rabbits/cage had no measurable adverse effects on the welfare of male and female young rabbits, but 5 in this example did.

References

Barnard CJ, JM Behnke & *J Sewell*: Social behaviour and susceptibility to infection in house mice (Mus musculus): effects of group size, aggressive behaviour and status-related hormonal responses prior to infection on resistance to Babesia microti. Parasitology 1994, *108*, 487-496.

Chu L, JP Garner & *JA Mench:* A behavioral comparison of New Zealand White rabbits housed individually or in pairs conventional laboratory cages. Appl Anim Behav Sci, 2004, *85*, 121-139.

CoE (Council of Europe): European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). Strasbourg: Council of Europe, 1986.

CoE (Council of Europe): Working Party for the preparation of the fourth Multilateral Consultation of Parties to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). Strasbourg: Council of Europe, 2004.

Dawson B & *RG Trapp*: Basic and clinical biostatistics. 3rd ed. Lange Medical Books/ McGraw-Hill Medical Publishing Division, New York, 2001.

Lehmann M: Interference of a restricted environment- as found in battery cages- with normal behaviour of young fattening rabbits. In Auxilia, T. (Ed), Rabbit production systems including welfare. CEC Agriculture, ECSC-EEC-EAEC, Brussels. 1987, 257-268.

Love JA & *K Hammond:* Group housing rabbits. Lab Animal, 1991, 20, 37-43.

Morisse JP & *R Maurice*: Influence of stocking density or group size on behaviour of fatteninig rabbits kept under intensive conditions. Appl Anim Behav Sci 1997, *54*, 351-354.

NRC (National Research Council): Guide for the Care and Use of Laboratory Animals. 7th Ed. Washington, DC: National Academy Press, 1996.

Perez C, JR Canal, E Dominguez, JE Campillo, M Guillen, MD Torres: Individual housing influences certain biochemical parameters in the rat. Lab Anim. 1997, *31*, 357-361.

Podberscek AL, JK Blackshawand & *AW Beattie:* The behaviour of group penned and individually caged laboratory animals. Appl Anim Behav Sci 1991, *28*, 353-363.

Simon J: Effects of daily corticosterone injection upon plasma glucose, insulin, uric acid and electrolytes and food intake pattern in the chicken. Diabetes Metab 1984, *10*, 211-217.

Trocino A, G Xiccato, PI Queaque & *A Sartori* : Group housing of growing rabbits: Effect of stocking density and cage floor on performance, welfare, and meat quality. Proc. 8th World Rabbit Congress, Mexico. 2004.

Turner RJ, DDE Held, JE Hirst, G Billinghurst & *RJ Wootton:* An immunological assessment of group housed rabbits. Lab Anim. 1997, *31*, 362-372.

Whary M, R Peper, G Borkowskı, W Lawrence & *F. Ferguson:* The effect of group housing on the research use of the laboratory rabbit. Lab Anim. 1993, *27*, 330-341.

Wolfensohn S & *M Lloyd*: Handbook of laboratory animal management and welfare. 3th ed, Blackwell Publishing, U.K. 2003.