Indian Journal of Experimental Biology
Vol. 63, January 2025, pp. 103-111
DOI: 10.56042/ijeb.v63i01.10615

Impact of cage density on body weight, organ mass, cortisol concentration, and blood metabolites in Wistar albino rats

Buket Boğa Kuru[1]* & Mustafa Makav[2]

[1]Department of Animal Breeding and Husbandry, [2]Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye

Received 22 April 2024; revised 09 September 2024

This study investigated the effects of different cage density and gender on growth performance, live weight gain, organ weight, cortisol concentration, and some biochemical parameters in growing Wistar rats. Twenty four, 6 weeks old rats (12 males, 12 females) were followed up to 15 weeks of age. The male and female control groups (MC and FC) were kept in cages at normal density, while the high cage density groups (MHD and FHD) were housed in cages at twice the density. Blood was collected and organs were weighed at 15 weeks of age. Live weight was significantly affected by both cage density and gender in male and female rats. Live weight was 7% lower in the MHD group than in the MC group in males and 9.5% lower in females. Heart, spleen, and total intestinal weights were lower in the MHD group than in the MC group in males. Serum cortisol, glucose, triglyceride, alanine aminotransferase, aspartate aminotransferase, and total protein concentrations were different between both MC and MHD and FC and FHD. Serum low-density lipoprotein and albumin concentrations were higher in MHD than in MC. In conclusion, high cage density negatively affects live weight, organ weights, and some biochemical parameters in growing Wistar rats.

Keywords: Housing density, Growth performance, Organ weight, Gender, Stress

Laboratory animals are instrumental in advancing scientific knowledge across a broad spectrum of research fields[1]. They contribute significantly to experimental studies, and the findings obtained from these animals are frequently extrapolated to inform the development of solutions for medical problems. However, this extensive reliance on animal models raises ethical concerns. An animal's response to its housing environment can be interpreted as an attempt to maintain physiological homeostasis and normal growth[2,3]. Providing an appropriate environment for animals to exhibit normal behaviors and adapt is of critical importance for both animal welfare and the reliability of experiments[4,5]. Therefore, it is necessary to design the experimental environment in a way that supports the comfort and natural behaviors of the animals. When comfort is not provided, animal growth and organ development can be adversely affected, which can reduce the reliability of experimental results. Additionally, it is known that a stressful environment can negatively impact animal health and well-being, and this can be demonstrated by parameters such as hematological parameters and hormone levels[6].

Inadequate housing conditions, particularly during extended durations, can elicit stress, distress, and negative affective states in laboratory rats. These conditions can manifest in various behavioral alterations, including anhedonia, anxiety, and aggression. Such alterations have been demonstrated to negatively impact the response to nociceptive stimuli, induce hyperalgesia, impair learning and memory consolidation, promote inter-animal aggression, and compromise the post-disease immune response. Therefore, mitigating chronic stress factors in research facilities is critical for ensuring the well-being of laboratory rats and the validity of experimental data. The stress response system encompasses the hypothalamic-pituitary-adrenal (HPA) axis and the locus coeruleus/noradrenergic system. Within the HPA axis, perceived threats are relayed to the hypothalamus, which subsequently triggers the release of corticotropin-releasing hormone (CRH) by neurons located within the paraventricular nucleus[7,8]. This study investigated the effects of high cage density and gender on growth performance, live weight gain, organ weight, serum cortisol concentration, and a selection of biochemical parameters in growing Wistar albino rats.

Materials and Methods

Animal and feed

Twenty-four, 6 weeks old Wistar albino rats (12 males and 12 females) were used in this study. After a 14-day adaptation period prior to the start of the study, the rats were housed in a controlled environment at 23 ± 1°C and 52 ± 3% humidity. All rats were exposed to a 12 h light/dark lighting cycle. The post-weaned rats were fed a specially prepared commercial standard pellet diet (metabolic energy 2600kcal/kg, crude protein 23%, Bayramoğlu Yem®, Erzurum, Türkiye, Table 1) to meet their nutritional

*Correspondence:
E-mail: buket_vetfak@hotmail.com

Table 1 — The content of pellet feed

Analytical		Additive		Trace elements components	
Humidity	12.80%	Vitamin A	12,000,000 IU/kg	Manganese (manganese sulfate)	86 mg/kg
Crude protein	23.00%			Iron (iron sulfate monohydrate)	31 mg/kg
Crude oil	3.70%			Zinc (zinc oxide)	96 mg/kg
Raw cellulose	7.70%			Cobalt (cobalt carbonate)	0.50 mg/kg
Raw clay	6.90%			Selenium (selenium selenite)	0.30 mg/kg
Sodium	0.50%			Iodine (calcium iodate anhydride)	2.20 mg/kg

Table 2 — Minimum floor areas (cm^2) of rats in groups according to weight

Bady weight (g)	Floor area per rat (cm^2) Group 1 and Group 2 [11]	Group 3 and Group 4
100-200 g	148	75
200-300 g	187	95
300-400 g	258	130
400-500 g	387	195

requirements[9]. Cages were cleaned twice a week, and rats were provided with water at all times *via* rubber-tipped glass bottles. Commercial pellet feed and drinking water were provided to the rats *ad libitum*.

Experimental design

Rats were divided into control (male = 6, female = 6) and high cage density (male = 6, female = 6) groups based on balanced body weights according to gender (Table 2). All rats were monitored until the age of 15 weeks. Before starting the study, vaginal cytology was performed as described in the literature to identify female rats in similar estrous cycles[10]. Due to the longer duration of the luteal phase compared to the follicular phase, rats in the diestrus stage were included in the study. Group 1–Male rats (Male control-MC, n = 6); Group 2–Female rats (Female control-FC, n = 6) were housed in cages of standard density according to the *Guide*[11]; Group 3–Male rats (Male high cage density-MHD, n = 6); Group 4–Female rats (Female high cage density-FHD, n = 6) were housed in cages of double density according to the *Guide*[11].

Body weight

Rat body weights were measured weekly using a scale with an accuracy of 0.1 grams (Desis®, Türkiye) until the age of 15 weeks.

Euthanasia

At the end of the 15 weeks period, rats were anesthetized with an intramuscular injection of xylazine (15 mg/kg, Rompun®, Bayer, Germany) and ketamine hydrochloride (75 mg/kg, Ketasol®, Richter Pharma AG, Austria). All rats were then euthanized by cervical dislocation. Blood was quickly collected, and organs were excised and weighed.

Blood collection

Blood samples (5-7 mL) were collected from the rats *via* cardiac puncture into vacuum tubes (BD Vacutainer® SST II Advance, Becton, Dickinson and Company, UK) and centrifuged at 3000 rpm for 10 min at 4°C using a centrifuge (NF 400R®, Nüve, Türkiye). After centrifugation, the obtained serum samples were stored at -18°C until biochemical analyses were performed.

Relative organ weights

Immediately following euthanasia, the heart, kidneys, liver, lungs, spleen, stomach, total intestine, brain, eyes, testes, ovaries, and uterus were collected. After excision, organs were separated from their surrounding tissues and weighed (Weightlab Instruments, Türkiye) immediately to three decimal places (bilateral organs were weighed together).

Biochemical analysis

Serum cortisol concentration was measured using a fully automated chemiluminescent immunoassay method on an ADVIA Centaur® XP (Siemens Healthcare Diagnostics, Tokyo, Japan) analyzer with a compatible commercial kit. Serum glucose, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), albumin, total protein, urea, and creatinine were measured using a fully automated photometric method on an Abbot® ARCHITECT (Abbott Diagnostics, Lake Forest, IL, USA) analyzer with commercial kits.

Statistical analysis

Data normality for each group was assessed using histograms and the Shapiro-Wilk test. Growth performance, daily live weight gain, organ weights, and biochemical parameters of the control groups were compared with the high cage density groups on weighing days using the Independent Samples *t-test*.

Table 3 — Gender-specific weekly growth performance (g) in Wistar albino rats subjected to varying cage density

Age (week)	Male	Mean ± SD	*P* value	Female	Mean ± SD	*P* value
6	Control	156.29 ± 12.35	NS	Control	140.43 ± 13.14	NS
	High density	157.43 ± 20.90		High density	141.43 ± 11.22	
7	Control	236.57 ± 20.54	NS	Control	174.00 ± 10.38	NS
	High density	235.57 ± 33.96		High density	172.00 ± 11.89	
8	Control	271.71 ± 12.82	NS	Control	198.71 ± 15.29	NS
	High density	265.86 ± 29.86		High density	195.14 ± 5.15	
9	Control	305.00 ± 19.55	NS	Control	207.14 ± 10.79	NS
	High density	302.43 ± 27.88		High density	205.57 ± 4.08	
10	Control	314.57 ± 24.21	NS	Control	213.71 ± 10.84	NS
	High density	316.43 ± 21.18		High density	209.57 ± 7.41	
11	Control	335.00 ± 19.71	NS	Control	222.29 ± 6.95	*
	High density	326.00 ± 17.16		High density	214.14 ± 3.24	
12	Control	351.29 ± 21.33	NS	Control	230.86 ± 6.89	**
	High density	339.00 ± 18.64		High density	219.57 ± 3.78	
13	Control	375.57 ± 17.72	NS	Control	237.29 ± 5.09	***
	High density	357.43 ± 18.68		High density	223.57 ± 5.59	
14	Control	385.29 ± 18.39	*	Control	247.86 ± 8.23	***
	High density	360.86 ± 13.83		High density	228.71 ± 5.94	
15	Control	401.00 ± 23.37	*	Control	254.57 ± 7.72	***
	High density	373.86 ± 15.28		High density	230.57 ± 2.76	

[SD: Standard deviation, NS: Not significant. Asterisks indicate statistical differences, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$]

The effects of cage density and gender on live weight, organ weights-percentages, and biochemical parameters, as well as the gender × cage density interaction, were determined using General Linear Model (GLM). Data are presented as mean ± standard deviation (SD). GraphPad Prism® (Version 9.5.1, GraphPad Software Inc., San Diego, CA, USA) and SPSS® (Version 26.0, SPSS Inc./IBM Group, Chicago, IL, USA) were used for statistical analysis. Statistical significance was set at $P < 0.05$ for group comparisons.

Results and Discussion

Live weight changes

Female rats were significantly affected by high cage density starting at 11 weeks of age, while male rats were not affected until 14 weeks of age (Table 3). At the end of the study, body weights in the MC and MHD groups (control and high density, respectively) were 401.00 ± 23.37 g and 373.86 ± 15.28 g ($P < 0.05$). Similarly, female body weights in the FC and FHD groups (control and high density) were 254.57 ± 7.72 g and 230.57 ± 2.76 g, respectively ($P < 0.001$, Table 3).

In terms of daily live weight gain, male rats were not statistically affected by high cage density stress compared to the control group ($P > 0.05$). However, in females, daily live weight gain was statistically higher in FC than FHD at 15 weeks of age ($P < 0.05$, Table 4).

While both gender ($P < 0.001$) and high cage density ($P < 0.001$) had significant effects on live weight in Wistar albino rats, there was no significant interaction between gender and density (Table 5).

Relative organ weights

Organ weights in Wistar albino rats were significantly affected by gender ($P < 0.01$), with the exception of the eyes. High cage density had a significant effect on heart ($P < 0.001$), spleen ($P < 0.01$), and total intestinal weight ($P < 0.001$). Uterus weight was statistically higher in FC compared to FHD ($P < 0.001$, Table 5), as was total intestinal weight ($P < 0.01$, Fig. 1). Conversely, liver ($P < 0.01$) and spleen ($P < 0.05$) weights were lower in FC compared to FHD (Fig. 1). Additionally, total intestinal weight was statistically higher in MC compared to MHD ($P < 0.01$, Fig. 1). The effects of high cage density and gender on organ weight percentage in Wistar albino rats are presented in Table 6.

Blood metabolites

Gender had a significant effect on serum LDL ($P < 0.01$) and AST ($P < 0.05$). High cage density had a statistically significant effect on other blood metabolites, except for serum urea and creatinine (Table 7). There was also a significant gender × high cage density interaction ($P < 0.05$) for serum albumin concentration (Table 7). Additionally, serum cortisol,

Table 4 — Daily live weight gain (g) in Wistar albino rats subjected to different cage density

Age (week)	Male	Mean ± SD	*P* value	Female	Mean ± SD	*P* value
7	Control	11.47 ± 4.29	NS	Control	4.79 ± 1.51	NS
	High density	11.16 ± 2.84		High density	4.37 ± 0.54	
8	Control	5.02 ± 3.06	NS	Control	3.53 ± 1.40	NS
	High density	4.33 ± 3.57		High density	3.31 ± 1.60	
9	Control	4.76 ± 2.37	NS	Control	1.20 ± 0.93	NS
	High density	5.22 ± 3.69		High density	1.49 ± 0.70	
10	Control	1.37 ± 1.36	NS	Control	0.94 ± 0.51	NS
	High density	2.00 ± 1.08		High density	0.57 ± 0.46	
11	Control	2.92 ± 1.14	*	Control	1.23 ± 0.68	NS
	High density	1.37 ± 0.75		High density	0.65 ± 0.56	
12	Control	2.32 ± 0.99	NS	Control	1.22 ± 0.58	NS
	High density	1.85 ± 1.35		High density	0.77 ± 0.49	
13	Control	3.47 ± 1.82	NS	Control	0.92 ± 0.51	NS
	High density	2.63 ± 0.88		High density	0.57 ± 0.44	
14	Control	1.39 ± 1.36	NS	Control	1.51 ± 0.85	NS
	High density	1.10 ± 0.49		High density	0.73 ± 0.63	
15	Control	2.24 ± 1.50	NS	Control	0.96 ± 0.21	*
	High density	1.24 ± 0.80		High density	0.27 ± 0.45	

[SD: Standard deviation, NS: Not significant. Asterisks indicate statistical differences, *: $P < 0.05$]

Table 5 — The effects of high cage density and gender on body weight and organ weights in Wistar albino rats

Body weight and organs	Gender		High cage density		*P* value		
	Male (Mean ± SD)	Female (Mean ± SD)	Male (Mean ± SD)	Female (Mean ± SD)	Gender	HD	Gender × HD
Body weight (g)	401.00 ± 23.37	254.57 ±7.72	373.86 ± 15.28	230.57 ± 2.76	***	***	NS
Heart (g)	1.16 ± 0.03	0.83 ± 0.03	1.24 ± 0.02	0.87 ± 0.02	***	***	NS
Kidney (g)	1.56 ± 0.03	1.12 ± 0.06	1.58 ± 0.02	1.10 ± 0.03	***	NS	NS
Liver (g)	12.91 ± 0.59	8.10 ± 0.10	13.16 ± 0.48	8.36 ± 0.16	***	NS	NS
Lung (g)	2.10 ± 0.08	1.96 ± 0.38	2.26 ± 0.11	1.83 ± 0.08	**	NS	NS
Spleen (g)	1.06 ± 0.16	0.70 ± 0.05	1.19 ± 0.09	0.81 ± 0.11	***	**	NS
Stomach (g)	1.96 ± 0.08	1.24 ± 0.09	1.93 ± 0.06	1.32 ± 0.08	***	NS	NS
Total intestine (g)	26.40 ± 0.93	18.62 ± 0.44	24.90 ± 0.49	17.86 ± 0.67	***	***	NS
Brain (g)	1.98 ± 0.04	1.75 ± 0.05	1.94 ± 0.06	1.76 ± 0.04	***	NS	NS
Eyes (g)	0.276 ± 0.014	0.274 ± 0.017	0.279 ± 0.022	0.277 ± 0.015	NS	NS	NS
Testes (g)	1.44 ± 0.11	-	1.38 ± 0.08	-	-	NS	-
Ovary (g)	-	0.073 ± 0.010	-	0.067 ± 0.006	-	NS	-
Uterus (g)	-	0.665 ± 0.037	-	0.519 ± 0.029	-	***	-

[HD: High cage density, SD: Standard deviation, NS: Not significant. Asterisks indicate statistical differences, **: $P < 0.01$, ***: $P < 0.001$]

glucose, triglyceride, ALT, AST, and total protein were statistically different between both MC and MHD, and FC and FHD (Fig. 2). Serum LDL ($P < 0.05$) and albumin ($P < 0.01$) concentrations were higher in MHD compared to MC (Fig. 2).

Rodents, particularly Wistar albino rats, have emerged as a foundational model organism in diverse scientific research fields. Their compact size facilitates efficient husbandry and maintenance within laboratory environments. Additionally, their relatively short reproductive cycles enable the rapid establishment of research cohorts. Moreover, advancements in genetic engineering techniques have expanded the utility of rats in unraveling intricate biological processes. This is achieved through the creation of strains harboring specific genetic modifications[12,13]. One critical factor influencing animal welfare within research settings is cage density. Crowded housing conditions can exert detrimental effects on rodent behavior and physiology, potentially compromising stress and health parameters. Studies have emphasized the significance of cage design and the number of cohabitating animals in mitigating stress levels. Consequently, determining optimal stocking densities requires meticulous consideration of these factors to

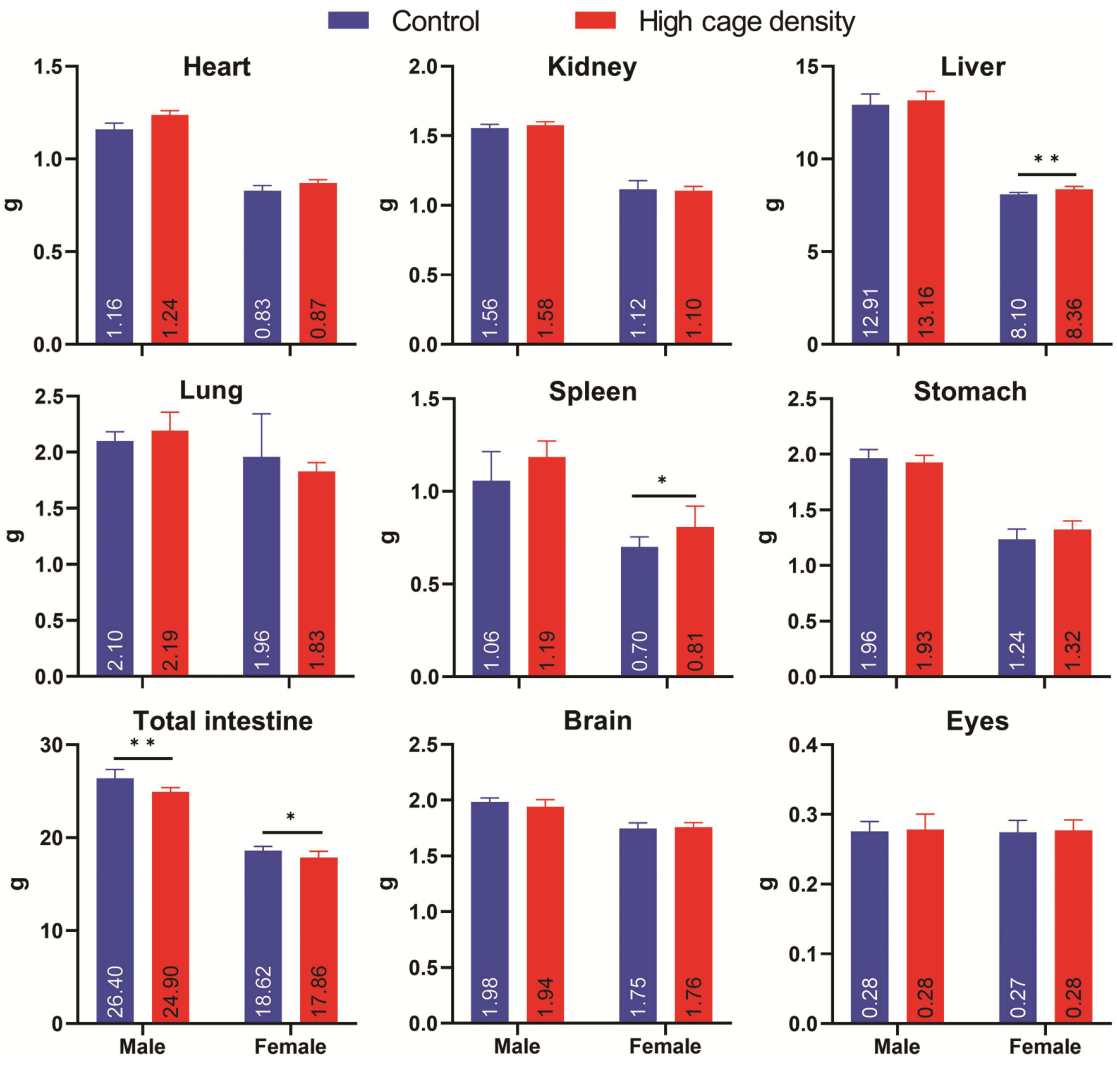

Fig. 1 — Effect of cage density on relative organ weights by gender. [Asterisks indicate statistical differences, *: $P < 0.05$, **: $P < 0.01$]

guarantee the ethical care of laboratory animals[14,15]. The present study was designed to investigate the effects of high cage density on growth performance, live weight gain, organ weights, serum cortisol concentration, and specific blood metabolites in Wistar albino rats.

Stocking density plays a critical yet often overlooked role in the welfare of experimental animals during production. A study investigating the effects of different cage types and densities on mice found no significant differences in growth rates, food, or water consumption[16]. Another study examined the impact of growth and stocking density on stress parameters in rats, revealing no adverse effect on body weight gain[17]. However, the mean weight of rats housed at high density was lower compared to those in standard or medium density cages. When feed and water intake were assessed over 6 weeks, no statistically significant differences were observed between standard, medium, and high-density groups[15]. Similarly, Wistar albino rats exposed to different stress models for 1 and 2 weeks, respectively, exhibited significantly reduced body weight changes[18]. Exposure to stress in Lewis rats has been found to decrease body weight and food intake while increasing anxiety-like behaviors and hyperactivity[19]. Additionally, various stressors in mice have been shown to increase locomotor activity, body weight loss, and induce anxiety associated with oxidative stress[20]. In our study, continuous high cage density exposure also affected growth performance in Wistar albino rats. The effect on body weight started to manifest after 9 weeks in male rats and 6 weeks in female rats. Notably, both gender and high cage

Table 6 — Effects of high cage density and gender on organ weight percentage in Wistar albino rats

Organs	Gender		High cage density		*P* value		
	Male (Mean ± SD)	Female (Mean ± SD)	Male (Mean ± SD)	Female (Mean ± SD)	Gender	HD	Gender × HD
Heart (%)	0.29 ± 0.01	0.32 ± 0.02	0.33 ± 0.01	0.38 ± 0.01	***	***	NS
Kidney (%)	0.39 ± 0.02	0.44 ± 0.03	0.42 ± 0.02	0.48 ± 0.02	***	***	NS
Liver (%)	3.23 ± 0.20	3.18 ± 0.12	3.53 ± 0.16	3.62 ± 0.08	NS	***	NS
Lung (%)	0.53 ± 0.03	0.77 ± 0.14	0.61 ± 0.05	0.79 ± 0.04	***	NS	NS
Spleen (%)	0.26 ± 0.05	0.28 ± 0.03	0.32 ± 0.03	0.35 ± 0.05	NS	***	NS
Stomach (%)	0.49 ± 0.03	0.52 ± 0.02	0.49 ± 0.05	0.57 ± 0.03	*	***	*
Total intestine (%)	6.60 ± 0.32	7.31 ± 0.14	6.67 ± 0.24	7.75 ± 0.31	***	*	NS
Brain (%)	0.49 ± 0.03	0.69 ± 0.03	0.52 ± 0.01	0.76 ± 0.02	***	***	*
Eyes (%)	0.07 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.12 ± 0.01	***	**	NS
Testes (%)	0.36 ± 0.04	-	0.37 ± 0.02	-	-	NS	-
Ovary (%)	-	0.029 ± 0.004	-	0.029 ± 0.002	-	NS	-
Uterus (%)	-	0.26 ± 0.02	-	0.23 ± 0.01	-	**	-

[HD: High cage density, SD: Standard deviation. NS: Not significant. Asterisks indicate statistical differences, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$]

Table 7 — Effects of high cage density and gender on serum cortisol and blood metabolites in Wistar albino rats

Metabolites	Gender		High cage density		*P* value		
	Male (Mean ± SD)	Female (Mean ± SD)	Male (Mean ± SD)	Female (Mean ± SD)	Gender	HD	Gender × HD
Cortisol (ug/mL)	0.13 ± 0.04	0.14 ± 0.04	0.23 ± 0.06	0.22 ± 0.04	NS	***	NS
Glucose (mg/dL)	86.00 ± 11.57	85.28 ± 11.88	146.28 ± 25.52	162.57 ± 22.69	NS	***	NS
Triglyceride (mg/dL)	52.14 ± 8.41	49.43 ± 15.03	71.43 ± 11.15	70.43 ± 8.58	NS	***	NS
HDL (mg/dL)	19.66 ± 2.08	21.56 ± 3.61	26.58 ± 10.52	25.06 ± 5.32	NS	*	NS
LDL (mg/dL)	11.20 ± 0.92	7.84 ± 0.99	22.74 ± 12.17	10.57 ± 2.70	**	**	NS
ALT (U/L)	15.71 ± 1.25	14.57 ± 2.88	23.57 ± 3.45	21.86 ± 3.58	NS	***	NS
AST (U/L)	68.71 ± 7.67	58.43 ± 20.39	93.86 ± 6.98	83.14 ± 9.72	*	***	NS
GGT (U/L)	1.29 ± 0.48	1.86 ± 0.38	2.29 ± 1.25	2.42 ± 1.13	NS	*	NS
Albumin (mg/dL)	1.58 ± 0.22	1.78 ± 0.47	2.47 ± 0.45	2.02 ± 0.37	NS	**	*
Total protein (mg/dL)	2.06 ± 0.12	2.25 ± 0.76	3.44 ± 0.99	4.12 ± 1.17	NS	***	NS
Urea (mg/dL)	32.29 ± 2.06	30.85 ± 7.17	32.85 ± 5.37	34.85 ± 8.63	NS	NS	NS
Creatinine (mg/dL)	0.30 ± 0.05	0.26 ± 0.04	0.29 ± 0.05	0.25 ± 0.04	NS	NS	NS

[HD: High cage density, HDL: High density lipoprotein, LDL: Low density lipoprotein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, SD: Standard deviation, NS: Not significant. Asterisks indicate statistical differences, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$]

density had significant effects on body weight in Wistar albino rats, which is consistent with the literature[21]. The observed body weight loss could be associated with changes in physiological responses to chronic stress in experimental animals. Moreover, decreased food intake in stressed rats may contribute to the reduction in body weight[18,22].

Female Wistar rats exhibit pronounced changes in internal organ weights when exposed to various stressors. For instance, stressors can induce a loss in the weight of the kidney, liver, and brain, particularly[18]. Restraint stress has also been reported to reduce uterine and ovarian weights[23]. High cage density during the growth phase has been reported to lead to significant reductions in the weights of certain internal organs in rats. Notably, marked decreases in heart, liver, and ovarian weights have been observed, with these effects being more pronounced in female rats[24]. Wistar rats exposed to different types of stressors have been found to exhibit significantly reduced kidney weights compared to the control group[25]. Similarly, increasing cage density in Sprague Dawley rats has been observed to cause reductions in heart, liver, kidney, testis, and ovary weights[3]. In this study, high cage density was found to have a significant effect on the weights of the heart, kidney, total intestine, and uterus. Notably, FHD rats had significantly larger liver and spleen weights compared to FC rats. These findings suggest that stress affects females more than males in terms of the corresponding organ weights. Therefore, continuous exposure to stressors can adversely affect organ functions and lead to metabolic and functional problems.

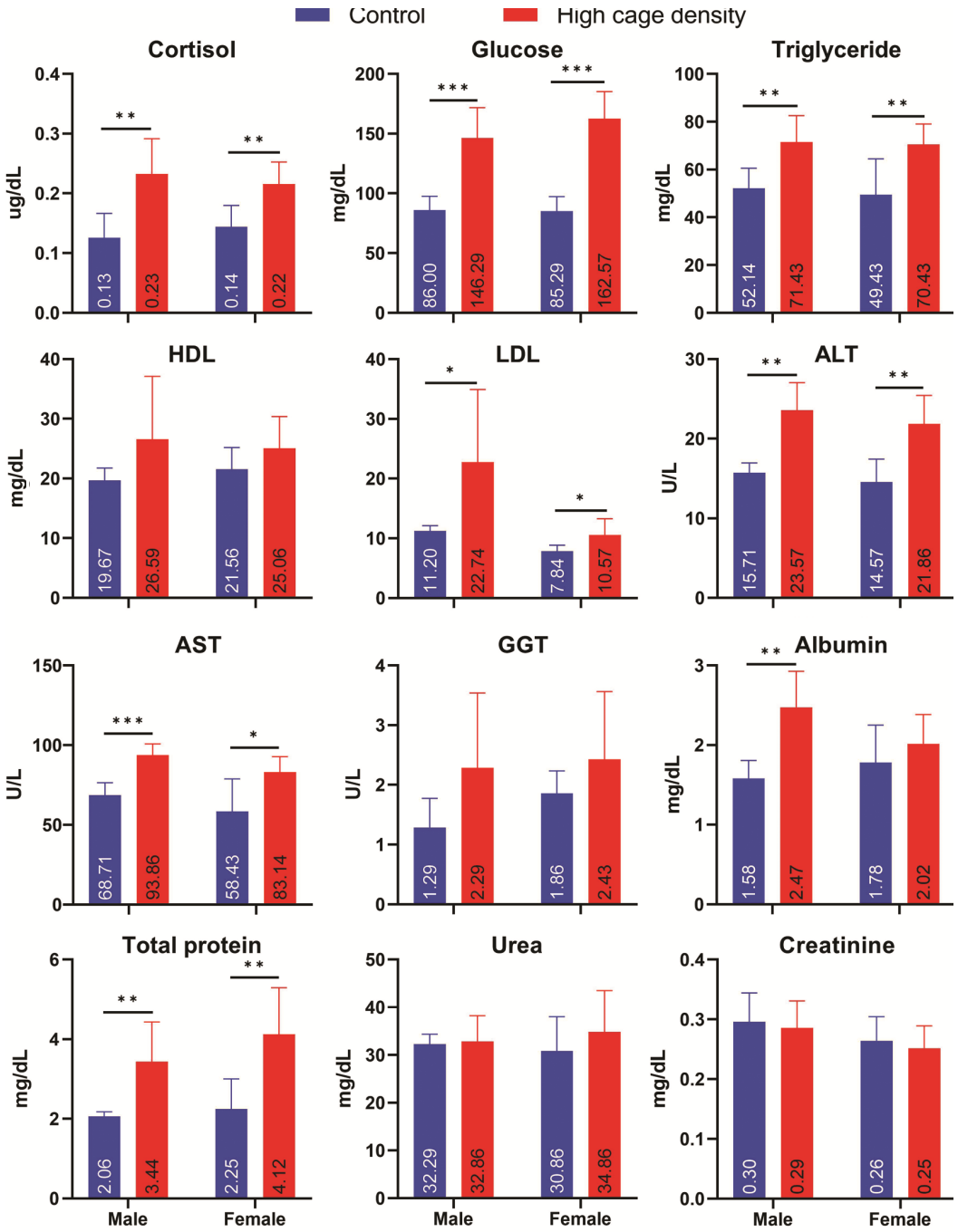

Fig. 2 — Effect of cage density on serum cortisol concentration and blood metabolites by gender. [Asterisks indicate statistical differences, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, HDL: High density lipoprotein, LDL: Low density lipoprotein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase]

Increased plasma corticosteroid levels have been observed in rats exposed to various stress stimuli[26,27]. Male rats adapt rapidly to hormonal imbalances caused by chronic stress factors, and their corticosteroid levels do not elevate[15]. However, males housed in crowded conditions may exhibit higher corticosteroid levels than females[28]. A study investigating social stress demonstrated that females exhibit higher corticosteroid levels compared to male rats in response to the stressor[29]. Similarly, female rats kept at high densities have higher plasma corticosteroid levels than those kept at standard densities. Male rats are not affected by high density in terms of plasma corticosteroid levels[15]. In this study,

there was no gender effect on cortisol concentration. However, the effect of high-density cages on serum cortisol concentration was highly significant. Additionally, both male and female rats housed in high-density cages showed higher cortisol concentrations compared to the control groups. Therefore, we believe that exposure to continuous high-density cage stress in Wistar albino rats can similarly affect the gender factor.

Blood glucose concentration in rats appears to be influenced by cage density, with males exhibiting lower concentrations compared to females[3]. Conversely, total protein and albumin concentrations were solely dependent on gender. Housing conditions, including cage density and size, can impact the blood chemistry of rats. Interestingly, female rats displayed more pronounced differences in three blood parameters (triglycerides, creatinine, and calcium) compared to males. These gender-based disparities were attributed to the varied responses of female and male rats to limited physical activity[24]. Exposure to three different stressors in Wistar albino rats did not significantly alter serum creatinine concentrations. While a 3-week exposure regimen to these stressors did not induce significant changes in serum creatinine, the authors hypothesized that a longer exposure period could potentially lead to further increases in concentration[25]. Additionally, serum urea concentration was suggested to vary depending on the response to different stress factors. In this study, gender did not significantly impact many blood metabolites. However, the effect of high cage density on blood metabolites was highly significant. Only urea and creatinine concentrations remained unaffected by cage density. A general increase in blood metabolites was observed within stress groups, with a particularly noteworthy rise in energy metabolism and liver profile indicators. These findings suggest that exposure to high cage density or crowded environments can induce physiological and metabolic alterations, leading to changes in specific serum biochemical parameters. Therefore, the measurement of such metabolites could be a valuable tool for monitoring the vital activities of organisms.

This study has several limitations. Firstly, using only Wistar albino rats may restrict the applicability of the findings to other rodent strains. Additionally, the study's duration might not reflect long-term effects of high cage density. Other factors, such as environmental enrichment and handling, were not fully controlled. The analysis was limited to a few blood metabolites, and a broader range could provide more insights. Moreover, the study did not address potential impacts on behavior or reproductive outcomes. Future research should include diverse strains, longer exposure periods, and additional physiological and behavioral parameters.

Conclusion

In conclusion, this study provides compelling evidence that high cage density has detrimental consequences for various physiological parameters in developing Wistar albino rats. Our findings demonstrate that high density significantly reduces body weight and the weights of vital organs like the heart, spleen, and total intestine, regardless of gender. Furthermore, it exacerbates stress by elevating serum cortisol and negatively impacts a range of biochemical parameters, including serum glucose, triglyceride, ALT, AST, and total protein. These observations highlight the critical need to optimize cage density and meticulously evaluate the housing conditions of experimental animals according to their growth and developmental stages.

Ethical statement

Ethical approval for this study was obtained from the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2024-12). Furthermore, all procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1 Domínguez-Oliva A Hernández-Ávalos I Martínez-Burnes J Olmos-Hernández A Verduzco-Mendoza A & Mota-Rojas D. The importance of animal models in biomedical research: Current insights and applications. *Animals*, 13 (2023) 1223.

2 Festing S & Wilkinson R. The ethics of animal research. Talking Point on the use of animals in scientific research. *EMBO Rep*, 8 (2007) 526.

3 Yıldız A Hayirli A Okumuş Z Kaynar Ö & Kısa F. Physiological profile of growing rats: Effects of cage type and cage density. *Asian-Australas J Anim Sci*, 20 (2007) 263.

4 Harris RBS Zhou J Youngblood BD Rybkin II Smagin GN & Ryan DH. Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. *Am J Physiol*, 275 (1998) R1928.

5 Li B Wang Y Rong L & Zheng W. Research progress on animal environment and welfare. *Anim Res One Health*, 1 (2023) 78.

6 Giral M Armengol C & Gavaldà A. Physiologic effects of housing rats in metabolic cages. *Comp Med*, 72 (2022) 298.

7 Pinelli C Leri F & Turner P. Long term physiologic and behavioural effects of housing density and environmental resource provision for adult male and female Sprague Dawley rats. *Animals*, 7 (2017) 44.

8 Aguilera G Nikodemova M Wynn PC & Catt KJ. Corticotropin releasing hormone receptors: Two decades later. *Peptides (NY)*, 25 (2004) 319.

9 NRC. *Nutrient Requirements of Laboratory Animals*. Fourth Edition. (National Academies Press, Washington, D.C.), 1995 doi:10.17226/4758.

10 Boğa Kuru B Makav M Yildiz G Ölmez M Kuru M Bektaşoğlu F Beşeren H Şahin T & Kırmızıbayrak T. The effect of *Ferula communis* L. on body-relative organ weight, serum and tissue oxidative status, biochemical and pathological changes in rats exposed to continuous light. *Kafkas Univ Vet Fak Derg*, 30 (2024) 363.

11 NRC. *Guide for the Care and Use of Laboratory Animals*. Eighth Edition. (National Academies Press, Washington, D.C.), 2011 doi:10.17226/12910.

12 Clarkson JM Martin JE & McKeegan DEF. A review of methods used to kill laboratory rodents: Issues and opportunities. *Lab Anim*, 56 (2022) 419.

13 Suckow MA & Wilson RP. *The Laboratory Rat*. Third Edition. (Academic Press, London), 2020 doi:10.1016/C2017-0-01188-6.

14 Hurst JL Barnard CJ Tolladay U Nevision CM & West CD. Housing and welfare in laboratory rats: Effects of cage stocking density and behavioural predictors of welfare. *Anim Behav*, 58 (1999) 563.

15 Calisir M Yilmaz O Kolatan HE & Sezgin AK. Effects of litter size and caging on physical and mental development in rats. *Physiol Behav*, 267 (2023) 114200.

16 Smith AL Mabus SL Stockwell JD & Muir C. Effects of housing density and cage floor space on C57BL/6J mice. *Comp Med*, 54 (2004) 656.

17 Genç M. The effects on the growth performance, some serum oxidative and nitrosative stress parameters of the stocking density in the Sprague-Dawley rats. *KSU J Agric Nat*, 23 (2020) 1359.

18 Nwogueze BC Ojieh AE Aloamaka CP Igweh JC & Onyesom I. Organ and body weights changes in female Wistar rats exposed to different stressors. *Nigerian Journal of Science and Environment*, 18 (2020) 135.

19 Berton O Aguerre S Sarrieau A Mormede P & Chaouloff F. Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience*, 82 (1997) 147.

20 Kumar A Kaur G & Rinwa P. Buspirone along with melatonin attenuates oxidative damage and anxiety-like behavior in a mouse model of immobilization stress. *Chin J Nat Med*, 12 (2014) 582.

21 Bean K Nemelka K Canchola P Hacker S Sturdivant RX & Rico PJ. Effects of housing density on Long Evans and Fischer 344 rats. *Lab Anim (NY)*, 37 (2008) 421.

22 Liliana González-Torres M Valerio C Santos D & Liliana Gonz Alez-Torres M. Uncontrollable chronic stress affects eating behavior in rats. *Stress*, 22 (2019) 501.

23 Liu G Dong Y Wang Z Cao J & Chen Y. Restraint stress alters immune parameters and induces oxidative stress in the mouse uterus during embryo implantation. *Stress*, 17 (2014) 494.

24 Yıldız A Hayirli A Okumus Z Kaynar Ö & Kısa F. Physiological profile of juvenile rats: Effects of cage size and cage density. *Lab Anim (NY)*, 36 (2007) 28.

25 Nwogueze BC Ofili IM Nnama TN & Aloamaka CP. Oxidative stress-induced by different stressors alters kidney tissue antioxidant markers and levels of creatinine and urea: the fate of renal membrane integrity. *Sci Rep*, 13 (2023) 13309.

26 Khazen T Hatoum OA Ferreira G & Maroun M. Acute exposure to a high-fat diet in juvenile male rats disrupts hippocampal-dependent memory and plasticity through glucocorticoids. *Sci Rep*, 9 (2019) 1.

27 Chakraborty P Datta S McEwen BS & Chattarji S. Corticosterone after acute stress prevents the delayed effects on the amygdala. *Neuropsychopharmacology*, 45 (2020) 2139.

28 Brown KJ & Grunberg NE. Effects of housing on male and female rats: Crowding stresses males but calms females. *Physiol Behav*, 58 (1995) 1085.

29 Kant GJ Lenox RH Bunnell BN Mougey EH Pennington LL & Meyerhoff JL. Comparison of stress response in male and female rats: Pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology*, 8 (1983) 421.