KSÜ Tarım ve Doğa Derg 23 (5): 1359-1365, 2020
KSU J. Agric Nat 23 (5): 1359-1365, 2020
DOI:10.18016/ksutarimdoga.vi.681294

The Effects on the Growth Performance, Some Serum Oxidative and Nitrosative Stress Parameters of the Stocking Density in the Sprague-Dawley Rats

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ABSTRACT

Objective of his studywas to compare the growth performances of Sprague-Dawley female rats fed at normal and narrowed stocking density with certain stress parameters. 72 Sprague-Dawley female rats aged 4 weeks were used in the experiment. The average live weight per trial for normal and narrow stock density groups was calculated as 54.22 ± 2.65 and 55.53 ± 2.66 g. Animals' weekly weighs during a month indicated that as their weight increased, the floor area per animal gradually expanded. Female rats reach sexual maturity at 8-9 weeks. For this reason, the experiment was continued until the animals were 2 months old. Based on the weekly weighing results of the rats, it was found that live weights increased regularly for both groups and growth performances did not change according to stocking density (P>0.05). It was concluded that serum Total Antioxidant Capacity (TAC) and Glutathione (GSH) values were affected by the stocking density and that significantly reduced in the rats fed at narrowed stocking density. On the other hand, Total Oxidant Capacity (TOC), Nitric Oxide (NO) and malondialdehyde (MDA) values increased approximately 1.5 times in the rats at the group of narrowed stocking density. As a result, it was found that even if the increasing stocking density has no negative effect on the growth performance of the animals, it causes some physiological changes in blood values by stressing the animals. Therefore, in order to get the most accurate results in scientific studies, it must be paid attention to breed the animals at the ideal housing conditions.

Research Article

Article History
Received : 28.01.2020
Accepted : 14.03.2020

Keywords
Stocking density
Sprague-Dawley rats
Growth performance
Oxidative and nitrosative stress

Yerleşim Sıklığının Sprague Dawley Ratlarda Büyüme Performansı, Bazı Serum Oksidatif ve Nitrosatif Stres Parametreleri Üzerine Etkisi

ÖZET

Bu çalışma, normal ve dar yerleşim sıklığında yetiştirilen dişi Sprague-Dawley ratların büyüme performanslarının ve bazı stres parametrelerinin karşılaştırılması amacıyla yapılmıştır. Denemede 4 haftalık yaştaki 72 adet Sprague Dawley cinsi dişi rat kullanılmıştır. Normal ve dar yerleşim sıklığı grupları için deneme başı canlı ağırlık ortalamaları 54.22±2.65 ve 55.53±2.66 g olarak hesaplanmıştır. Hayvanlar 1 ay boyunca haftalık olarak tartılmış ve ağırlıkları arttıkça hayvan başına düşen taban alanları tedrici olarak artırılmıştır. Dişi ratlar, 8-9. haftada cinsel olgunluğa eriştiği için, deneme hayvanlar 2 aylık oluncaya kadar sürdürülmüştür. Ratların haftalık tartım sonuçlarına göre canlı ağırlıkların her iki grup için düzenli bir artış gösterdiği ve büyüme performanslarının yerleşim sıklığına göre değişmediği tespit edilmiştir (P>0.05). Serum Toplam Antioksidan Kapasite (TAC) ve Glutatyon (GSH) değerlerinin yerleşim sıklığından etkilendiği ve dar yerleşim sıklığında yetiştirilen ratlarda önemli ölçüde azaldığı sonucuna varılmıştır. Toplam Oksidan Kapasite (TOC), Nitrik oksit (NO) ve Malondialdehit (MDA) değerlerinin ise dar yerleşim sıklığı grubundaki ratlarda yaklaşık 1.5 kat arttığı belirlenmiştir. Sonuç olarak artan stoklama yoğunluğunun

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 28.01.2020
Kabul Tarihi : 14.03.2020

Anahtar Kelimeler
Yerleşim sıklığı
Sprague-Dawley sıçan
Büyüme performansı
Oksidatif ve nitrosatif stres

hayvanların büyüme performansı üzerine olumsuz bir etkisi olmasa bile, hayvanları strese sokarak kan değerlerinde bir takım fizyolojik değişikliklere yol açtığı saptanmıştır. Bu sebeple bilimsel çalışmalarda en doğru sonucun elde edilebilmesi için hayvanların mutlaka ideal barındırma koşullarında yetiştirilmiş olmasına dikkat edilmelidir.

To Cite : Genc M 2020. 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 (5): 1359-1365. DOI: 10.18016/ksutarimdoga.vi.681294.

INTRODUCTION

Today, most of the scientific researches in the field of health is carried out on experimental animals (Ergun, 2011). Because of their several advantages such as to easily adapt to the laboratory environment, to be raised easily, to grow rapidly, to rapidly grow in sexual maturity, to be close to human for genetic, biological, and behavioral characteristics, besides, their small physical structures and their cheap prices; rats are considered as the most suitable model of mammal system in the late 18th or early 19th century (Sengupta, 2013; Tufek ve Ozkan, 2018; Uludag, 2019). These animals have become the most widely used experimental animal in biomedical and behavioral research especially in the last 80 years (Sengupta, 2013; Saruhan and Dereli, 2016).

In order to obtain reliable results from experimental scientific studies and to succeed in experimental methods developed, starting with healthy animals is an indispensable scientific rule. Production conditions take an important place in obtaining healthy animal. The excitement, discomfort or chronic stress on animals caused by any negative effects may lead some morphological, physiological, biochemical, psychological, and behavioral changes and cause, therefore, causing errors in the experimental results. This, on the other hand, reduces the reliability of the experiment (Palanza, 2001; Barker et al., 2017).

The welfare of the animal has importance in terms of protecting the rights of experimental animals commonly used in experiments and ensuring the reliability of the data obtained. For the maintenance and care of the laboratory animals raised for scientific purposes, international standards have been put into action. The Guide for the Care and Use of Lab Animals (United States) (Council, 2010) and the EC Directive 2010 / 63 / EU (Europe) (EU Directive, 2010) are two different legal regulation setting the standards of raising the rodent animals. In order to obtain reliable results in scientific studies where conventionally raised laboratory animals have been used, the animals should be healthy and be raised in accordance with these standards (Ergun, 2011; Okur, 2016; Uludag, 2019).

One of the most important factors that affect the welfare of the laboratory animal and cause chronic stress in them is the space allowances per animal. Rats, living in groups within social status in their natural living environments, can be housed in groups also conventionally. Therefore, the presence of similar and optimal number of animals around the rats raised in cages positively affects their welfare. However, high stocking density, applied in order to benefit more effectively from the unit area, leads to undesirable effects, such as limiting the locomotion of animals and changing their microenvironment (humidity, temperature and air quality deterioration in cages) (Hurst et al., 1997; Arakawa, 2005; Yildiz et al., 2007; Barker et al., 2017). This situation, where the offspring is more sensitive, causes weakening of the immune status of the animals and leads them to be more susceptible to the harmful effects of environmental factors (Hurst et al., 1999; Arakawa, 2005; Yildiz et al., 2007; Ergun, 2011; Barker et al., 2017; Benjamin, 2019). Pursuant to the legislation of the Guide for the Care and Use of Lab Animals (United States), recommended minimum floor area for the rats weighed lighter than 100 g is 109.6 cm^2, while it is 148.35 cm^2 for the rats weighed up to 200 g, besides, the height of the cage should not be less than 17.8 cm (Council, 2010). In accordance with the legislation of EC Directive 2010/63/EU (Europe), on the other hand, a minimum 200 cm^2 floor area per animal should be allocated for the rats with the live weight up to 200 g and the height of the cage should not be less than 18 cm (EU Directive, 2010).

This aim of this study was to investigate the growth performances of the Sprague-Dawley female rats raised in a high stocking density conditions and the changes in some stress parameters.

MATERIALS and METHODS

The ethics committee approval was obtained from the Office of Animal Experiments Local Ethics Committee at Atatürk University at 27.06.2019 (7/106/2019), following that, the study was carried out at the Medical Experimental Application and Research Center. Sprague-Dawley female weaner rats aged 4 weeks were separated in control and test groups of 6 rats for each, a total of 72 rats were used in the study by six repetition. The average live weight per trial for normal and narrow stock density groups was calculated as 54.22 ± 2.65 and 55.53 ± 2.66 g. The animals were housed in the cages (min. vertical height 18 cm, length

44.5 cm and width 34 cm) in a room under a constant controlled temperature at 21-24 °C with a constant 12-h light-dark cycles. Besides, rats were fed with mixed feed containing 16% crude protein and 2700 kcal/kg metabolic energy. Feed and water was provided *ad libitum*.

Control Group (CG): The cage floor space was determined according to the normal stocking density (approximately 100 cm² for the rats lighter than 100 g, approximately 200 cm² for the rats weighed between 100-200 g, and approximately 250 cm² for the rats weighed between 200-300 g).

High Stocking Density (HSD): The floor space was calculated according to the high stocking density (an arrangement was made as to be approximately 75% of the control group).

The rats were weighed weekly for a month and the floor spaces per animal were arranged according to the average live weight of the rats in the cage. The cages were divided by grids and stocking densities were increased gradually as the animals grew.

Biochemical Analysis

After 24-h following the end of 1-month-test period, the serums were taken from the blood samples taken from the rats decapitated under the sevoflurane anesthesia, after that, were analyzed in terms of TAC, TOC, NO, GSH and MDA. Serum TAC (Erel, 2004) and TOC (Erel, 2005) were determined using the measurement methods developed by Erel (TAC and TOC assay kit, Rel Assay Diagnostic). NO measurement was determined by the enzymatic conversion of nitrate by nitrate reductase to nitrite followed by colorimetric detection of nitrite, a colored azo dye formed from the Griess-type reactions, which absorbs visible light at 540 nm (NO detection kit, Enzo Life Science). Changes in serum MDA levels were measured spectrophotometrically according to the method modified by Placer et al. (1966). Serum GSH content was determined at 412 nm by the methods of Sedlak and Lindsay (1968).

Statistical Analysis

The effect of stocking density on the growth performance of the rats and serum levels of TAC, TOC, NO, GSH, MDA was evaluated by Independent Sample T-Test using SPSS Package (SPSS, 2013).

RESULTS

Based on the weekly weighing results of the rats, it was found that live weights (LW) increased regularly for both groups and growth performances did not change according to stocking density (P>0.05, Figure 1).

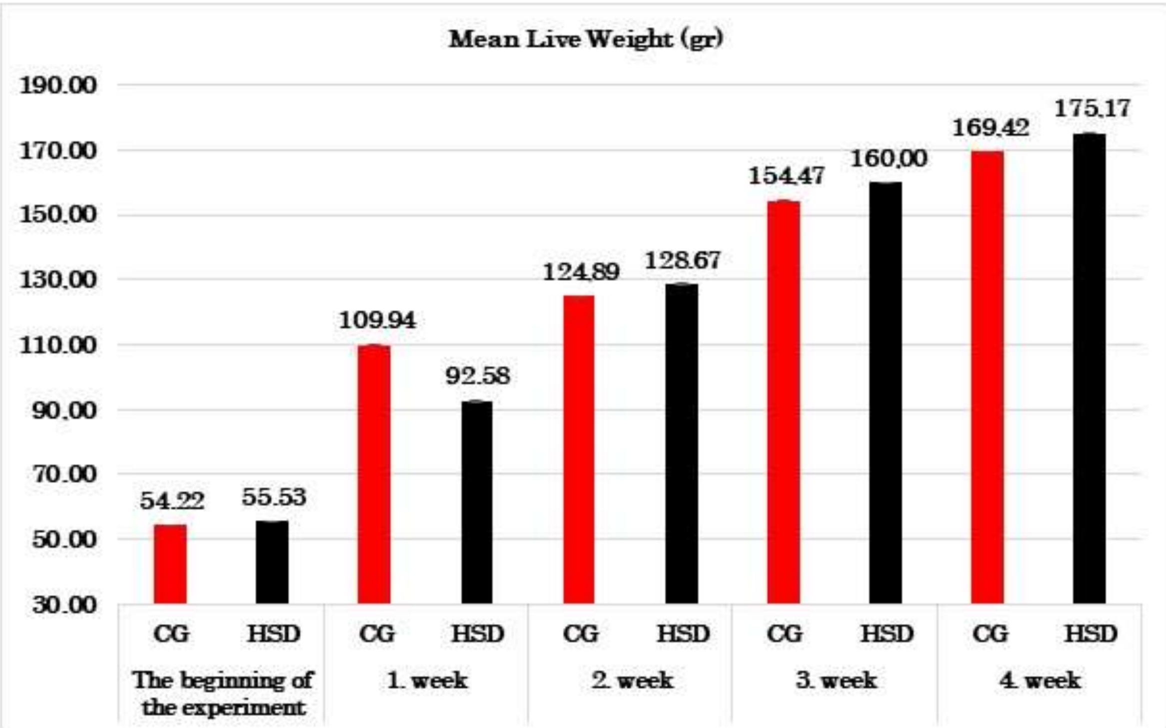

Figure 1: Weekly growth performances for the normal and high stocking density groups
Şekil 1: Normal ve yüksek stoklama yoğunluk grupları için haftalık büyüme performansları

TAC value was found to be (P<0.01) in the rats in the control group; while TOC value was found to be higher in rats raised at high stocking density (P<0.0001). NO, a widespread signaling molecule that participates in virtually every cellular and takes part in the defense system against intracellular pathogens, was found to increase approximately 1.5-fold in rats in the high stocking density group compared to the control group (P<0.0001). GSH, a non-enzymatic antioxidant, was found to be significantly reduced in the high stocking density group (P<0.0001). Serum MDA measurement value, which is a frequently used test for determining the degree of lipid peroxidation, was found to be 2.91 and 4.43 nmol/ml in the control and high stocking density groups, respectively; it was found that this value increased significantly by decreasing the cage floor space per animal (P<0.05), (Table 1).

Table 1: Serum TAC, TOC, NO, GSH and MDA results for normal and high stocking density groups
Tablo 1: Normal ve yüksek stoklama yoğunluk grupları için serum TAC, TOC, NO, GSH ve MDA sonuçları

	Treatment Uygulama	*Mean Ortalama*	*Std. Error Mean Ortalamanın Standart Hatası*	P
TAC mmol/L	CG	3.05	0.07	0.001
	HSD	2.65	0.05	
TOC µmol/L	CG	3.24	0.09	0.0001
	HSD	4.63	0.19	
NO µmol/L	CG	81.87	5.63	0.0001
	HSD	125.33	4.92	
GSH nmol/ml	CG	1.45	0.03	0.0001
	HSD	0.82	0.03	
MDA nmol/ml	CG	2.91	0.12	0.033
	HSD	4.43	0.61	

CG: Control Group, **HSD:** High Stocking Density

DISCUSSION and CONCLUSION

Recently, the most important source of biomedical research conducted in many fields including neuro-behavioral studies, and cancer and toxicology are the rats. Although it is difficult to assess the exact number of animals used in scientific experiments, it was estimated that approximately 15 million rats are used in the USA, 11 million in Europe, 5 million in Japan and 2 million in Canada (Alves and Colli, 2006). In experiments with laboratory animals that are not raised under appropriate conditions, there is a possibility that the correct results cannot be obtained. This condition negatively affects the reliability of the test results, leading losses of labor, time and animals (Palanza, 2001; Barker et al., 2017). Stocking density is considered as one of the most important conditions of breeding experimental animals. This criterion is often overlooked. Having investigated the effects of the stocking density on the growth performances of the rats and certain stress parameters, this study found that the increases of body weight (BW) in rats were not adversely affected by the stocking density. Similarly, there are different trials that conclude that cage stocking density does not change the growth performance in laboratory animals (Smith et al., 2004; Arakawa, 2005; Whitaker et al., 2007; Sen, 2015). Contrary to the findings, Michel et al. (2005) reported that stress factors in rodents stimulate the catabolic effect and lead to live weight loss; while Yildiz et al. (2007) stated that the increase in stocking density in Sprague-Dawley rats had negative effects on both sexes, however, females were more affected. On the other hand, Bean et al. (2008) argued that the rates of being affected by the stocking density for the rats vary according to races and that the increase of the live weight of the Long-Evans race was suppressed by being more affected by the mentioned stress factor. Gamallo et al. (1986) stated that growth performance slowed down dramatically if stocking density was doubled; while Smith et al. (2005) on the other hand, reported that although the stocking density varies according to genotypes in rodents, it negatively affects growth performance. The differentness in the findings obtained from the studies is thought to be due to differences in rats used in terms of race, gender, genetic structure, number of offspring at birth and floor space per animal.

Although there are different studies investigating the effects of the stocking density on various behavioral and physiological changes in rodents (Arakawa, 2005;

Smith et al., 2005; Whitaker et al., 2007), domestic hens (Simsek et al., 2014; Eugen et al., 2019), quail (Ayasan et al., 2000; Toplu and Fidan, 2008; Bahsi et al., 2016), fishes (Jia et al., 2016; Rayhan et al., 2018), pigs (Cornale et al., 2015; Kim et al., 2016; Larsen et al., 2018), broiler (Qaid et al., 2016; Li et al., 2019) and primates (Yamagiwa, 1999; Duncan et al., 2013), no studies have investigated its effect on antioxidant capacity. In the present study, therefore, the effect of the stocking density on the antioxidant capacity in the rats was examined. In some other studies, on the other hand, it was revealed that some toxic agents, chemicals and drugs reduce the TAC and GSH levels in serum and different tissues in the rats; while they increase the TOC, MDA and GSH levels (El-Tantawy, 2016; Benzer et al., 2018; Caglayan et al., 2018).

When the antioxidant defense system is impaired, body cells and tissues become more susceptible to dysfunction and/or diseases. Therefore, maintaining adequate levels of antioxidants is necessary to prevent and control multiple disease states (Kusano and Ferrari, 2008). MDA, one of the first reactions of the body against oxidant balances in living organisms, is one of the most important markers of the lipid peroxidation indicator. Increased MDA changes antioxidant balance by activating antioxidants to prevent damage. However, as a result of the long struggle, it is known that antioxidants lost this war and the damage started to increase. In the study, it was found that stocking density stress increased MDA levels in rats (Celi, 2010; Sharma and Bist, 2018). According to the results, it is understood that oxidants increase and begin to damage the cell membrane. It is not possible for cells with impaired membranes to survive and perform their functions. Glutathione (GSH), a tripeptide synthesized in the liver, is one of the body's first lines of defense against damage to cell membranes as a non-enzymatic antioxidant. The body counteracts the oxidant damage caused by antioxidant defense systems and one of the first attack steps in this is GSH. However, when GSH stores are not reinforced and depleted, cell damage increases gradually (Sharma and Bist, 2018). In the study, it is thought that in animals in the high stocking density group, the GSH level decreases due to depletion of GSH stores. TAC, which is one of the parameters that reflect the oxidant balance in the body in general, expresses the antioxidant effect potential of a living thing against oxidant agents (Atakisi et al., 2016). TAC level in this study was decreased in the rats in the high stocking density group. When looking at the TOC level, which generally expresses the oxidant balance of the body, it was increased and oxidant balance was dominant in the body of stressed rats and the antioxidant balance was weakened. NO is an important biomarker in determining the body's nitrosative balance (Atakisi et al., 2016). The increase of NO levels in rats in the high stocking density group was in full agreement with the MDA and TOC parameters we obtained in living organisms. Certain studies suggested that the floor space per animal may be reduced up to half of the recommended size without causing significant negativity (Smith et al., 2005; Nicholson et al., 2009; Barker et al., 2017). However, in the present study, the stocking density was found to be effective on antioxidant components of rats, and it was determined that oxidative stress occurs in rats raised at high stocking density and therefore harmful effects were observed; on the other hand, normal stocking density was found to reduce the oxidative stress and chemical toxicity.

Accurate and reliable data of scientific studies can only be obtained from healthy animals. Therefore, physically and physiologically healthy animals should be used in such the experiments. As a result, although the rats exposed to the stress of stocking density were found to be healthy in their macroscopic examinations, their oxidant-antioxidant balance was impaired. Experiments with animals that were thought to be healthy but whose antioxidant balance was impaired causing insecurity in the data. Such insecurity would cause deviation in values, false positivity or negativity from the first day of starting the study. Researchers cannot evaluate the results obtained from these rats and most importantly, they will not be able to support them with scientific data and literature. This situation causes time, labor and financial losses. Therefore, it was concluded that the stocking density should be taken into consideration in the selection of rats used in scientific studies.

ACKNOWLEDGEMENTS

The author acknowledges Prof. Dr. Fatih Mehmet KANDEMIR for biochemical analysis.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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