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# Haematological, biochemical and oxidative stress parameters of New Zealand rabbits housed at different stocking densities

## Parámetros hematológicos, bioquímicos y de estrés oxidativo de conejos de Nueva Zelanda mantenidos en diferentes densidades de población

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**ABSTRACT**

The aim of this study was to investigate the effects of different stocking densities on oxidative stress, some hematological and biochemical blood parameters in New Zealand rabbits, and to determine the ideal cage area where rabbits can live without being stressed. A total of 36 1-month-old mixed sex New Zealand weaned rabbits were selected for the study. The rabbits were placed in cages at different densities of stocking. Experimental groups were 1 rabbit per cage ($C_1$), 2 rabbits per cage ($C_2$) and 3 rabbits per cage ($C_3$). There was no significant differences between groups in terms of RBC, HGB, HCT, MCV, MCH, WBC, neutrophils, lymphocytes, monocytes, eosinophlis, basophlis, AST, ALT, trigliseride, cholesterol, HDL and LDL parameters ($P$>0.05). $C_2$ group had lower count of PLT comparing $C_1$ and $C_3$ groups. $C_1$ group had the highest value for MCHC ($P$<0.05). As oxidative stress parameters, there was no significant differences between IMA and TAS parameters ($P$>0.05). $C_2$ group had the lowest value in terms of SOD ($P$<0.05). In conclusion $C_2$ group was determined more advantageous in terms of breeding and welfare, since $C_1$ group does not support social needs of the animals and $C_3$ group leads to increased stress levels due to reduced individual space and restricted movement.

**Key words:** Blood parameters; oxidative stress; rabbit; SOD; stocking density; TAS

**RESUMEN**

El propósito de este estudio fue investigar los efectos de diferentes densidades de población en conejos de Nueva Zelanda sobre el estrés oxidativo, así como algunos parámetros sanguíneos hematológicos y bioquímicos, con el objetivo de determinar el área ideal de la jaula donde los conejos puedan vivir sin estar estresados. Se utilizaron un total de 36 conejos destetados de Nueva Zelanda, con una edad de 1 mes, como material animal. Estos conejos fueron distribuidos en jaulas con diferentes densidades de población, siendo los grupos experimentales: 1 conejo (C1), 2 conejos (C2) y 3 conejos (C3) por jaula. No se observaron diferencias significativas entre los grupos de densidad de población en términos de parámetros como RBC, HGB, HCT, MCV, MCH, WBC, neutrófilos, linfocitos, monocitos, eosinófilos, basófilos, AST, ALT, triglicéridos, colesterol, HDL y LDL ($P$>0,05). Sin embargo, se encontró que el grupo C2 presentaba un recuento más bajo de plaquetas (PLT) en comparación con el grupo C1 y el grupo C3. Además, el grupo C1 mostró el valor más alto de MCHC ($P$<0,05). En cuanto a los parámetros de estrés oxidativo, no se observaron diferencias significativas entre los grupos en los parámetros IMA y TAS ($P$>0,05). No obstante, el grupo C2 presentó el valor más bajo en términos de actividad de la superóxido dismutasa (SOD) ($P$<0,05). En conclusión, se determinó que la densidad de población representada por el grupo C2 era más ventajosa en términos de reproducción y bienestar animal. Esto se debió a que el grupo C1 no satisfacía las necesidades sociales de los animales, mientras que el grupo C3 conducía a niveles más altos de estrés debido al espacio individual reducido y al movimiento restringido.

**Palabras clave:** Parámetros sanguíneos; estrés oxidativo; conejo; SOD; densidad de población; TAS

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## INTRODUCTION

Blood, a crucial part of the circulatory system, consists of cells suspended in plasma and plays a fundamental role in maintaining homeostasis. While hematological parameters provide insights into the physiological responses of the animal to various stimuli; biochemical parameters serve as valuable indicators of the health and function of internal organs and systems [1, 2, 3].

Stress is a biological response in the form of anatomical, physiological and behavioral changes that threaten the homeostasis. Animals experience varying degrees of both psychological and physiological stress at different stages of their lives. To mitigate the risk of excessive stress, it is crucial to employ objective measures for identifying its causative factors. Properly managing an animal's exposure to stress factors not only has a positive impact on their productivity but also enhances overall welfare [4].

In animal production, the stress level is one of the important criteria used to determine the welfare. Stress arises from the influence of various factors, and it is recommended to analyze a combination of criteria, including productivity levels, behavioral characteristics, and physiological parameters (hematological, enzymatic, and hormonal), in order to accurately identify the causes of stress. This integrated approach enables a comprehensive understanding of the factors impacting animal welfare and facilitates effective strategies for stress management in animal production systems [5, 6, 7].

Ovuru and Ekweozor [8] emphasized that blood data was a very important indicator in understanding whether animals were adapted to the environment in which they were raised. Environmental changes can induce stress in animals, leading to alterations in blood parameters as a result of physiological responses to these environmental challenges. Monitoring blood parameters provides valuable insights into the adaptive capacity of animals and helps in understanding how they respond to and cope with changes in their surroundings. This emphasizes the role of blood analysis as a key tool in assessing the overall welfare and adaptability of animals to their environment. New Zealand rabbit (*Oryctolagus cuniculus*) stands out with its short generation interval, high fertility rate and short gestation period. Indeed, rabbits serve a dual role as both laboratory animals commonly used in experimental studies and important farm animals recommended for breeding. One of the key factors contributing to the significance of rabbits in farming is the nutritional composition of their meat. Rabbit meat is known for its lower fat content, higher levels of omega–3 fatty acids, polyunsaturated fatty acids, and essential amino acids. In terms of the superior properties of the meat, it is among the functional foods whose importance has been increasing in the health sector in recent years [9, 10].

The establishment of standard conditions in laboratory animal breeding is important for the uniformity and reliability of the results to be obtained at the end of the study. In this respect, it is necessary to provide animals convenient environment that meet their requirements in which they can adapt without geting stressed. At the same time, animals that adapt to different conditions easily and remain unaffected by the environmental changes contribute to the enhanced reliability of study results. Providing a suitable and stress–free environment not only promotes the welfare of laboratory animals but also plays a vital role in obtaining accurate and reproducible scientific data [2, 11, 12].

Increasing the quantity of animals raised per unit area in farm conditions can lead to enhanced profitability, as labor and costs per animal decrease. Commercial enterprises often strive to maximize the number of animals raised per unit area to optimize efficiency. While this approach can be advantageous for overall production, it is crucial to recognize that higher animal stocking density, especially in confined spaces like cages, may result in increased stress for the animals. This heightened stress can potentially lead to more frequent occurrences of aggressive and dominant behaviors among the animals [13, 14].

The ideal cage size for rabbits should provide enough width for the animal to stretch its body and stand straight simultaneously In addition, it should be taken into account that young rabbits need more space due to their rapid movements and higher activity This ensures that the living environment accommodates their natural behaviors and allows for proper physical development. Providing adequate space in cages is not only essential for the rabbits' welfare but also contributes to their overall health and well–being [15].

The study aimed to investigate the effects of different stocking densities on oxidative stress, some hematological and biochemical blood parameters in New Zealand rabbits. Additionally, the study aimed to identify the optimal cage area that allows rabbits to live without experiencing stress. By assessing these factors, the research sought to contribute valuable insights into the relationship between stocking density and the physiological welfare of New Zealand rabbits, ultimately aiming to inform practices that promote a healthier and less stressful living environment.

## MATERIAL AND METHODS

### Ethical statement

The study was accepted by the Balikesir University Animal Experiments Local Ethics Committee (2020/4–19).

### Experimental design and animals

The study was carried out at Balikesir University Experimental Animals Research Center. 18 male and 18 female totaly 36 one month old New Zealand rabbits were used in the study. The weaned rabbits were separated from their mothers, placed into experimental cages and the study was started. Each rabbit was assigned a number, and each rabbit was randomly transferred to the experimental cages according to the simple random selection method. The low sexual dimorphism and the early age of rabbits justify the standard practice of mixed sex rearing of females and males in intensive commercial farms and laboratories. The research period was lasted 2 months. During this period, a commercial ration was prepared in accordance with the needs of the rabbits was given ad libitum and provide water in drinkers at all times of experiment. Animals were fed an experimental ration appropriate for their energy and protein requirements. Ingrediants and chemical composition of the experimental diet were shown in TABLE I

### Housing conditions of the rabbits

Animals were placed in stainless steel racks with plastic suspension cages at different stocking densities. The cages in which the animals housed were standard and their dimensions were 71.3 × 71.6 × 47.6 cm (width×depth×height). Experimental groups were 1 rabbit per cage ($C_1$), 2 rabbits per cage ($C_2$) and 3 rabbits ($C_3$) per cage. The floor area

*TABLE I*
**Ingredients and chemical composition of the experimental diet**

| **Ingredients** | **(%)** |
|---|---|
| Barley | 20.75 |
| Corn | 18.00 |
| Wheat Bran | 14.00 |
| Soybean meal | 18.50 |
| Yeast | 0.20 |
| Alfalfa flour | 25.00 |
| Methionine | 0.15 |
| Phytase | 0.10 |
| L–lysine | 0.20 |
| By–pass fat | 1.50 |
| Vitamin and mineral mixture[1] | 1.60 |
| **Chemical composition** | **(%)** |
| Dry matter | 94.20 |
| Crude protein | 19.35 |
| NDF | 31.45 |
| ADF | 14.55 |
| ADL | 1.02 |
| Ether extract | 4.45 |
| Starch | 19.50 |
| Ash | 9.68 |
| Digestible energy (kcal·kg$^{-1}$) | 2,850 |

NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin. [1]Supplied per kg diet: 13,000 IU vitamin A; 1200 IU vitamin D3; 75 mg vitamin E; 2 mg vitamin $B_1$; 6 mg vitamin $B_2$; 3 mg vitamin $B_6$; 0.02 mg vitamin $B_{12}$; 0.3 mg Co; 8 mg Cu; 27 mg Fe; 19 mg Mn; 44 mg Zn; 0.07 mg Se

allocated for each rabbit was determined as 0.43 m$^2$ for the group of 1 rabbit housed in the cage, 0.21 m$^2$ for the group of 2 rabbits housed in the cage and 0.14 m$^2$ for the group of 3 rabbits housed in the cage (FIGS. 1, 2 and 3).

**FIGURE 1. $C_1$ (1 rabbit / cage group)**


**FIGURE 2. $C_2$ (2 rabbits / cage group)**


**FIGURE 3. $C_3$ (3 rabbits / cage group)**

### Analysis of haematologic, biochemical and oxidative stress parameters

Haematological analysis was performed on the LH 780 (Beckman Coulter Inc., USA). WBC (white blood cell), RBC (red blood cell) and PLT (platelet) were measured with the coulter principle, and HGB (hemoglobin) was measured by photometric method. MCV (mean corpuscular volume) was obtained from the RBC histogram, while MPV (mean platelet volume) was obtained from the PLT histogram. HCT (hematocrit), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) were calculated from the measured blood parameters. The formulas were given in TABLE II.

Serum AST (aspartate aminotransferase), ALT (alanine aminotransferase), triglyceride, total cholesterol, HDL (high density lipoptotein) and LDL (low density lipoptotein) levels were analyzed by spectrophotometric method in an AU680 (Beckman Coulter Inc., USA) clinical chemistry autoanalyzer. Serum SOD (superoxide dismutase) activity (Elabscience Cat. No: E–BC–K020–M) and serum TAS (total antioxidant status) (Rel Assay Lot No: EL21121A) levels were studied by colorimetric method. Serum IMA (Ischemia–modified albumin) level was determined by enzyme–linked immunosorbent assay (ELISA) method (SunRed Cat. No: 201–1–1672).

***TABLE II***
**The formulas of the calculated blood parameters in New Zealand rabbits**

| Test | Formula |
|---|---|
| HCT | (RBC × MCV) / 10 |
| MCH | (HGB / RBC) × 10 |
| MCHC | (HGB / HCT) × 100 |

HCT Hematocrit; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration

### Statistical analysis

Data were evaluated by one way analysis of variance (ANOVA) in SPSS software version 25 [16]. Cage stocking density was the experimantal unit of the study. The significance of the differences were compared by Tukey test. $P<0.05$ was considered significant.

## RESULTS AND DISCUSSION

In the study, the research focused on investigating the effects of various stocking densities on oxidative stress, hematological, and biochemical blood parameters in New Zealand rabbits. Blood parameters serve as crucial indicators, offering insights into the animals' stress conditions, inflammation, necrosis, as well as potential infections or damage to visceral organs. The examination of these parameters is essential for comprehensively understanding the physiological responses and overall health status of the rabbits under different stocking densities [17].

Measurements of erythrogram parameters at different stocking densities of New Zealand rabbits were shown in TABLE III. There were no significant differences between stocking density group in terms of RBC, HGB, HCT, MCV, MCH parameters ($P>0.05$). $C_1$ group had the highest value for MCHC ($P<0.05$) and differences between $C_2$ and $C_3$ groups were not significant. $C_2$ group had lower count of PLT comparing $C_1$ and $C_3$ groups ($P<0.01$).

It has been revealed that RBC and HCT parameters were influenced by stress, age, gender, season and breed [18, 19]. HCT rate under 30% or decrease in HCT and HGB levels is typically considered indicative of anemia [19]. In the study, no significant differences were found between the stocking density groups in terms of RBC, HGB and HCT parameters. This lack of variation may be attributed to the similarity in rabbit body weights and ages across the groups [11]. At the same time, HCT and HGB values were among the reported reference values [1]. It was seen that there was no problem in terms of oxygen and carbon dioxide transport in tissues due to the function of hemoglobin. When under stressful conditions, the animals demonstrated resilience in terms of this parameter. Consistent with research findings on RBC, El–Bayoumi *et al.* [20] didn't find significant differences between different stocking density groups. Abdel Hamid [21] didn't report any differences for HGB. In contrast to researh findings, Kalaba *et al.* [22] reported the highest values of RBC and HGB for 2 rabbits reared per cage and 4 rabbits reared per cage group had the lowest values among stocking density groups. Another study, involving Californian and crossbred rabbits housed at different densities found that the group with 20 rabbits·m$^{-2}$ had the lowest RBC value, while the groups with 8 rabbits·m$^{-2}$ and 12 rabbits·m$^{-2}$ had higher values [21]. Omar *et al.* [12] informed that RBC of 24 rabbits·m$^{-2}$ group had the lowest value compared with 8 rabbits·m$^{-2}$ and 16 rabbits·m$^{-2}$ groups and there was no significant differences between 8 rabbits·m$^{-2}$ and 16 rabbits·m$^{-2}$ groups.

***TABLE III***
**Mean erythrogram parameters in different stocking density of New Zealand rabbits**

| Blood Parameters | $C_1$ (n=12) | $C_2$ (n=12) | $C_3$ (n=12) | SEM | *P* |
|---|---|---|---|---|---|
| RBC, 10$^3$ cell·µL$^{-1}$ | 6.04 | 6.34 | 6.17 | 0.09 | – |
| HGB, g·dL$^{-1}$ | 13.46 | 13.81 | 13.64 | 0.20 | – |
| HCT, % | 40.48 | 42.32 | 41.89 | 0.63 | – |
| MCV, fL | 66.92 | 66.80 | 67.24 | 0.35 | – |
| MCH, pg | 22.27 | 21.80 | 22.13 | 0.13 | – |
| MCHC, g·dL$^{-1}$ | 33.29$^a$ | 32.63$^b$ | 32.52$^b$ | 0.14 | * |
| PLT, 10$^3$ cell·µL$^{-1}$ | 364.33$^a$ | 212.42$^b$ | 297.33$^a$ | 20.79 | ** |

$C_1$: 1 rabbit per cage, $C_2$: 2 rabbits per cage $C_3$: 3 rabbits per cage. RBC: Red Blood Cell; HGB: Hemoglobin; HCT Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelet. SEM: Standard error of mean. –: $P>0.05$; *: $P<0.05$; **: $P<0.01$. $^{a,b}$: Values within a row with different superscript differ significantly at $P<0.05$

MCV, MCH and MCHC values are important parameters that allow to make evaluation of different haematological situations in the body and the amount of circulating erythrocytes. They play a significant role in the diagnosis of anemic conditions [23, 24]. In the study, there was no significant differences between the stocking density groups in terms of MCV and MCH values. The MCHC value was the highest in 1 rabbit reared per cage group in the study and an increase in the number of rabbits housed in the cage resulted a significant decrease in MCHC. Similar to the findings of this study, Omar *et al.* [12] found no significant differences for MCV and MCH, and Yakubu *et al.* [25] found no significant differences for MCV values under different stocking densities. On the other hand, Kalaba *et al.* [22] reported that 1 rabbit reared per cage group had the highest value of MCHC and 2 rabbits reared per cage, 3 rabbits reared per cage and 4 rabbits reared per cage groups had lower values than this value. In terms of MCV value, 3 rabbits reared per cage group had higher values than 5 rabbits reared per cage and 7 rabbits reared per cage groups for New Zealand rabbits [20]. In a different study conducted California rabbits, 2 rabbits reared per cage group had the highest value in terms of MCV and it was reported that 4 rabbits reared per cage group had the lowest values [22].

PLT cells have important roles in maintaining homeostasis, thrombus formation, atherosclerosis, inflammation and immune response. An increase or activation of the number is associated with a high thrombotic risk, while a decrease may be due to heredity or permanent damage [26]. In the study 1 rabbit reared per cage group had higher PLT values compared with 2 rabbits reared per cage group. The observed increase in the level of PLT could be associated with oxidative stress and cardiovascular diseases [27]. The lower value of 2 rabbits reared per cage group suggested that the animals in 1 rabbit reared per cage group could be stressed, as the increase in this value for the 3 rabbits reared per cage group supported this opinion. Trocino *et al.* [28] reported that rabbits housed individual cages had the highest level of fear comparing with collective cages. Because of social contacts of the rabbits housed in groups were in a less stressful condition. The fact that 2 rabbits reared per cage group had lower values in terms of PLT parameter showed that this group might not have experienced stress and adapted to the situation in terms of housing conditions. Similar to the research findings Aboegla

*et al.* [29] reported that while 2 rabbits reared per cage group had the lowest values of PLT; 1 rabbit reared per cage and 4 rabbits reared per cage groups had highest PLT values in New Zealand rabbits.

Leucogram parameters in different stocking density of New Zealand rabbits were presented in TABLE IV. There was no significant differences between stocking density groups in terms of WBC, neutrophils, lymphocytes, monocytes, eosinophils and basophils ($P$>0.05).

***TABLE IV***
**Mean leucogram parameters in different stocking density of New Zealand rabbits**

| Blood Parameters | $C_1$ (n=12) | $C_2$ (n=12) | $C_3$ (n=12) | SEM | *P* |
|---|---|---|---|---|---|
| WBC, $10^3$ cell·µL$^{-1}$ | 9.23 | 9.63 | 8.06 | 0,45 | – |
| Neutrophils, % | 25.85 | 23.18 | 19.40 | 1,84 | – |
| Lymphocytes, % | 68.06 | 70.19 | 76.93 | 2,06 | – |
| Monocytes, % | 4.10 | 3.03 | 1.81 | 0,49 | – |
| Eosinophils, % | 0.65 | 0.75 | 0.69 | 0,10 | – |
| Basophils, % | 1.28 | 2.86 | 1.17 | 0,74 | – |

$C_1$: 1 rabbit per cage, $C_2$: 2 rabbits per cage $C_3$: 3 rabbits per cage. WBC: White Blood Cell; SEM: Standard error of mean. –: $P$>0.05

WBC has an important role in the body's defense system against pathogens, forming the basis of the immune response. While high WBC count was generally associated with a microbial infection or presence of antigen; decreased WBC was seen in combat infections [2]. Keeping the WBC count in the normal ranges could be an indication that the immune system was active and sufficient. The ideal WBC of rabbits has been reported in the range of 4.5–11. The increase in the total WBC to 15–30 indicated that the rabbit may be under stress conditions [1]. In the study, it was determined that the housing system did not have a negative effect on WBC, as the values within different stocking densities remained within the ideal ranges. If WBC falled below the reference values, it might be associated with allergic conditions, anaphylactic shock or parasite infestations [1, 30, 31].

In accordance with the present findings, some studies have reported no significant differences in WBC counts between stocking density groups [12, 21]. Contrary to the findings of the study El–Bayoumi *et al.* [20] reported that as the stocking density increased, the WBC value increased and Aboegla *et al.* [29] informed that WBC count of 4 rabbits reared per cage group was the highest while 2 rabbits reared per cage group had the lowest levels.

When the leucogram parameters were examined in the study, it was seen that the stocking density did not affect these values. Neutrophil, lymphocyte, monocytes, and basophil counts were within normal ranges indicate the absence of inflammation, acute, chronic, or viral infections [32]. Similar to the research findings it was reported that neutrophil, lymphocyte, monocyte and eosinophil values examined in different stocking densities did not create a significant difference between groups. It was reported that high eosinophil value might be associated with allergic reactions or conditions [33]. In this regard, allergic reactions may be caused by housing factors due to hypersensitivity reactions. The absence of a significant difference between groups in terms of eosinophil values in the study indicates that there was no allergic situation among the groups. Similar to study findings there were no significant differences reported between stocking density groups to the count of monocytes, eosinophils, basophils [12] and lymphocytes [21].

Unlike the findings of the study, highest values were found in the 20 rabbits·m$^{-2}$ group in terms of lymphocyte count. It has been reported that the values in 12 rabbits·m$^{-2}$ and 8 rabbits·m$^{-2}$ groups continued to decrease and the lowest value was in the 8 rabbits·m$^{-2}$ group [21]. In another study, a significant decrease was observed in lymphocyte value as the stocking density increased [20]. It was reported that 4 rabbits reared per cage group had the highest values in terms of neutrophil count; 5 rabbits reared per cage groups and 3 rabbits reared per cage groups had lower values and reported that there were no significant difference between 3 rabbits reared per cage and 5 rabbits reared per cage groups [34].

Biochemical parameters of different stocking density groups were shown in TABLE V. Significant differences were not found between different stocking density groups in terms of AST, ALT, triglyceride, cholesterol, HDL and LDL ($P$>0.05).

***TABLE V***
**Mean biochemical parameters in different stocking density of New Zealand rabbits**

| Blood Parameters | $C_1$ (n=12) | $C_2$ (n=12) | $C_3$ (n=12) | SEM | *P* |
|---|---|---|---|---|---|
| AST, U·L$^{-1}$ | 15.67 | 15.92 | 21.67 | 2.16 | – |
| ALT, U·L$^{-1}$ | 27.83 | 38.00 | 48.83 | 5.03 | – |
| Triglyceride, mg·dL$^{-1}$ | 87.83 | 97.42 | 84.58 | 4.86 | – |
| Cholesterol, mg·dL$^{-1}$ | 106.00 | 78.33 | 97.50 | 6.93 | – |
| HDL, mg·dL$^{-1}$ | 36.50 | 30.58 | 35.83 | 1.85 | – |
| LDL, mg·dL$^{-1}$ | 68.17 | 47.75 | 60.92 | 5.17 | – |

$C_1$: 1 rabbit per cage, $C_2$: 2 rabbits per cage $C_3$: 3 rabbits per cage. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HDL: High density lipoptotein; LDL: Low density lipoptotein; SEM: Standard error of mean. –: $P$>0.05

In rabbits, serum biochemical parameters ensure important key factors about visceral organ damage, particulary in liver and kidneys [18, 35]. In the study, no significant differences were found in terms of the stocking density groups in terms of AST, ALT, triglyceride, cholesterol, HDL and LDL values. While there were no significant differences between different stocking density groups in terms of cholesterol, triglyceride, ALT and AST ratios similar to the findings of the study [12, 15, 34, 36, 37]. Kalaba *et al.* [22], Aboegla *et al.* [29] and El–Bayoumi *et al.* [38] stated that cholesterol and triglyceride values decreased as the stocking density increased. Contrary to the findings of the study, it was reported that the ALT value increased as the stocking density increased [22, 39]. In terms of HDL, the value obtained from 3 rabbits reared per cage group was found to be higher than the value obtained from 2 rabbits reared per cage group [37].

In normal physiological conditions, there exists a delicate equilibrium between the continuous formation of reactive oxygen species (ROS) within cells and the presence of antioxidants that counteract their effects. Disruption of this equilibrium in favor of an excess of ROS, along with the accumulation of superoxide radicals within the cell or an inadequate endogenous defense system, is referred to as oxidative stress. The increase in ROS is toxic to the cell and disrupts

the intracellular signaling mechanism by damaging proteins, lipids and nucleic acids in the cell. Antioxidants fundamentally work to prevent or delay cell damage by scavenging free radicals in the cell. SOD and TAS are among the important antioxidants in this context [39].

Ischemia–modified albumin (IMA) is a new marker of ischemia. It is a metabolic product of protein occurred during acute ischemic conditions or oxidative stress due to a decrease in binding capacity of albumin for transition metals such as iron, cobalt, copper and nickel [40, 41].

Oxidative stress parameters in different stocking density groups of New Zealand rabbits were shown in TABLE VI. There were no significant differences between IMA and TAS parameters ($P>0.05$). $C_2$ group had the lowest value in terms of SOD ($P<0.05$). There was no significant differences between $C_1$ and $C_3$ groups ($P>0.05$).

***TABLE VI***
**Mean oxidative stress parameters in different stocking density groups of New Zealand rabbits**

| Blood Parameters | $C_1$ (n=12) | $C_2$ (n=12) | $C_3$ (n=12) | SEM | *P* |
|---|---|---|---|---|---|
| IMA, U·mL$^{-1}$ | 79.58 | 84.83 | 85.83 | 2.14 | - |
| SOD, U·mL$^{-1}$ | 605.83$^{a}$ | 510.08$^{b}$ | 597.75$^{a}$ | 15.99 | * |
| TAS, mmol Trolox Equiv·L$^{-1}$ | 1.06 | 1.02 | 0.96 | 0.02 | - |

$C_1$: 1 rabbit per cage, $C_2$: 2 rabbits per cage $C_3$: 3 rabbits per cage. IMA: Ischemia modified albumin; SOD: Superoxide dismutase; TAS: Total antioxidant status; SEM: Standard error of mean. –: $P>0.05$; *: $P<0.05$. $^{a,b}$: Values within a row with different superscript differ significantly at $P<0.05$

Unlike study findings IMA levels were reported to increase significantly with prolonged ischemia a study conducted with New Zealand rabbits [40]. In the current study as the stocking density increased, the increase in the IMA value, although not statistically significant, can be interpreted as a response to stress.

In previous studies performed with New Zealand rabbits, were been reported that decrease in SOD and TAS levels might be associated with oxidative stress [42, 43]. In the study, SOD value determined in $C_1$ and $C_3$ groups was higher than the $C_2$ groups. It was suggested that individual struggle and fighting behaviors may have occurred among animals housed in $C_2$ group. Although it was not statistically significant, the level of TAS decreased as the stocking density increased. This suggests that the stress level might be increased as the animals' mobility and movement status decreased [28, 44].

## CONCLUSIONS

As a result of the research, in terms of blood MCHC value, $C_1$ group had higher values than $C_2$ and $C_3$ groups. As PLT value, $C_2$ group had lower values than $C_1$ and $C_3$ groups. It was determined that $C_2$ group had lower values in terms of SOD. According to the findings of the study, it can be said that the $C_2$ group was more advantageous in terms of breeding and welfare, since the $C_1$ group does not support the social needs of the animals, and thus increases the stress in the animals, and the animals adapt to these conditions more easily. In the $C_3$ group, animals tend to get stressed due to the decrease in the area per animal and the restriction of movement. In this respect, it is important to pay attention to the area per unit animal in cage breeding. When the findings of the study were evaluated, it was concluded that the $C_2$ group might be the ideal housing frequency in terms of cage breeding, where the animals can adapt without getting stressed, but the individual behaviors and struggle between the animals should be considered. In future studies, monitoring and evaluating the behavior of animals in cages and interpreting them together with blood results will support the research findings and contribute to the literature.

### Availability of data and materials

Data could be shared if requested from corresponding author (B. Yaranoğlu).

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### Conflict of interest

Authors declare that they have no conflict of interest.

### Author contributions

Experimental design was planned by BY, MHY, SU and AAH, animals care and management were performed by MHY, blood was collected by BY and MHY, blood analysis were performed by SU and AAH, statistical analysis were done by BY and SU, results were evaluated by BY, MHY, SU and AAH, manuscript was written by all authors contributed to the final version of the manuscript.

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