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Impact of Improved Animal Welfare on Hematological Parameters (Erythrocyte, Hemoglobin and Hematocrit) in Wistar Rats

Rini Amriani, Dian Fatmawati, Muhammad Dirga Gifardi, Muhammad Zulfadillah Sinusi, Muhammad Wahyudi Yusran, Dewi Mutiara, Magfira Satya Apada, Muhammad Danawir Alwi and Dwi Kesuma Sari

Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, 90245 Indonesia
***Corresponding author:** dwiks73@gmail.com

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ABSTRACT

The reliability of animal models in biomedical research, particularly for infectious and non-infectious diseases, hinges on their physiological and metabolic stability, coupled with stringent animal welfare. This study aimed to optimize Wistar rat (*Rattus norvegicus*) models by evaluating key physiological and metabolic parameters, specifically erythrocyte count, hemoglobin concentration, and hematocrit levels, under conditions prioritizing animal welfare. A total of 24 Wistar rats were divided into 8 groups, each subjected to distinct experimental conditions (aspects of housing management, environment, and nutrition) over 21 days. Blood samples were collected on the last day for hematology analysis. Data were analyzed using appropriate statistical methods, including normality, homogeneity, and Kruskal-Wallis tests where necessary. Initial findings indicate variations in erythrocyte, hemoglobin, and hematocrit levels across different groups, suggesting distinct physiological responses to experimental conditions. These results underscore the importance of comprehensive physiological monitoring and strict adherence to welfare principles in refining animal models, ensuring both scientific rigor and ethical practice in disease research.

Key words: Animal welfare, Erythrocyte, Hematocrit, Hemoglobin, Wistar rat.

INTRODUCTION

Animal models, particularly rodents, are indispensable in biomedical research for understanding the pathogenesis of various infectious and non-infectious diseases, as well as for evaluating the efficacy and safety of new therapeutic interventions (Voelkl et al. 2020). The success and validity of research findings derived from animal studies heavily rely on the optimal health, consistent physiological state, and minimized stress levels of the experimental animals. Routine handling and physical restraint procedures, though necessary for husbandry and experimental procedures, can significantly contribute to cumulative suffering and negatively impact both animal welfare and scientific outcomes. Physical restraint has been demonstrated to be aversive, causing negative affective states as well as cardiovascular and hormonal changes in mice, yet these regular handling interactions often receive little consideration despite their contribution to stress-related cumulative suffering (Davies et al. 2022; Xu et al. 2025).

Beyond handling procedures, conventional laboratory housing conditions themselves represent a significant source of chronic stress, as standard cages restrict the expression of natural behaviors such as burrowing, foraging, exploration, and climbing, leading to frustration, stereotypic behaviors, weakened immune responses, and increased anxiety in rodents (Ratuski and Weary 2022). Variations in physiological and metabolic parameters can significantly confound results, leading to misinterpretations and potentially flawed conclusions (Patel et al. 2024). Therefore, a robust understanding and meticulous monitoring of these parameters are critical for enhancing the reproducibility and translational relevance of animal research (Barros et al. 2018; Suresh et al. 2024; Patel et al. 2024). Infectious diseases continue to pose global health challenges, necessitating continuous research into their mechanisms and novel treatments.

Similarly, the increasing prevalence of non-infectious chronic diseases, such as metabolic disorders, cardiovascular conditions, and neurodegenerative diseases, demands reliable animal models for preclinical investigations. Rats have proven instrumental in studying

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diverse conditions, including stress-related disorders, infectious diseases, metabolic diseases, nutrition, immunology, neurology, and behavioral research due to their short developmental time, known genetic makeup, microbial status, and tractable nature. Their stress-free breeding, minimal animal handling and rearing care requirements, and minimal genetic variations make them particularly suitable for biomedical research, though they may not be ideal models for certain inflammation studies (Debnath et al. 2025; Lazăr et al. 2025). Wistar rats (*Rattus norvegicus*) are widely utilized due to their well-characterized genetics, ease of handling, and physiological similarities to humans in many aspects (Percie du Sert et al. 2020). However, the responsiveness of these models to disease induction and therapeutic agents can be influenced by their baseline physiological status and metabolic health, which are often reflected in hematological profiles (Kondashevskaia and Mkhitarov 2005). The health status, welfare, and productivity of nutrias under household rearing conditions can be significantly influenced by environmental and management factors, which are reliably indicated through their hematological and biochemical blood profiles (Lazăr et al. 2025). Hematology serves as an insightful diagnostic tool, revealing vital information regarding the health status, stress responses, and metabolic functioning (Firdaus et al. 2025).

Hematological changes occur, including a decrease in erythrocyte count and hematocrit alongside an increase in hemoglobin concentration and leukocyte levels. These alterations reflect the impact of glucocorticoids, key stress hormones, on the hematopoietic system, potentially disrupting red blood cell production and contributing to immune modulation. Erythropoietin may offer protective effects against such glucocorticoid-induced disruptions, helping to restore blood parameters and prevent exacerbated inflammatory responses (Hanssen and Iskander 2025). Hypoxia may arise under physiological conditions, such as high-altitude exposure, or from pathological states including anemia, cardiovascular disease, chronic obstructive pulmonary disease (COPD), neurodegenerative diseases, and chronic kidney disease. In these conditions, reduced erythrocyte function or count can lead to insufficient oxygen delivery, exacerbating tissue damage and metabolic disruptions (Xu et al. 2025).

Acute stress and elevated corticosterone levels can disrupt bone marrow stem cell function, leading to reduced production of blood cells such as erythrocytes. This alteration in hematological parameters may contribute to systemic inflammation and disease susceptibility (Xu et al. 2025). For instance, anemia (low erythrocyte count or hemoglobin) can impair tissue oxygenation, affecting cellular metabolism and the progression of disease processes or the efficacy of a therapeutic agent. Conversely, elevated hematocrit might indicate dehydration or other physiological disturbances (Voelkl et al. 2020; Debnath et al. 2025).

The normal ranges can signal underlying inflammation, infection, nutritional deficiencies, or stress-induced physiological alterations that may compromise the integrity of the disease model (Kamel et al. 2024; Xu et al. 2025).

Beyond scientific validity, the ethical imperative of animal welfare in research is paramount. The "3Rs" principle (replacement, reduction, refinement) guides contemporary animal experimentation, with "refinement" focusing on minimizing pain, distress, and improving the welfare of animals used (Kilkenny et al. 2010; Sena and Currie 2019). Optimizing animal models through comprehensive physiological evaluation, including hematological assessments, directly contributes to refinement by ensuring animals are in optimal health, thereby reducing variability, the number of animals needed (reduction), and ultimately enhancing the quality of scientific outcomes (Percie du Sert et al. 2020). Stress, for example, can alter hematological parameters and immune responses, thereby influencing disease progression and therapeutic outcomes (Guilherme et al. 2016). Consequently, assessing these markers under rigorous welfare standards provides a more accurate representation of the disease state and treatment effects (Kilkenny et al. 2010; Scarola et al. 2019).

This study aims to evaluate and optimize Wistar rat models for infectious and non-infectious disease research by systematically assessing their physiological and metabolic functions, with a particular focus on erythrocyte counts, hemoglobin concentration, and hematocrit levels. By integrating these hematological insights with robust animal welfare practices, we seek to provide a foundation for developing more reliable, reproducible, and ethically sound animal models that contribute meaningfully to biomedical advancements.

MATERIALS AND METHODS

Animals and housing

A total of 24 male Wistar rats (*Rattus norvegicus*), weighing 180-220g and approximately 8-10 weeks old, were obtained from the Biofarmasetics Laboratory, Faculty of Pharmacy, Hasanuddin University. They were provided with standard rodent chow and filtered water *ad libitum*. All animals underwent a 7-day acclimatization period before the start of the experiments to minimize stress.

Experimental design

Rats were randomly assigned to different experimental groups (aspects of housing management, environment, and nutrition) with 3 animals per group (Table 1). The duration of the experimental period for hematological assessment was 21 days.

Table 1: Group design with the specific treatment

Group	Treatment		
	Cage	Environment	Nutrition
K1	(++)	(*)	(##)
K2	(++)	(*)	(#)
K3	(++)	(**)	(##)
K4	(++)	(**)	(#)
K5	(+)	(**)	(#)
K6	(+)	(**)	(##)
K7	(+)	(*)	(#)
K8	(+)	(*)	(##)

Note:(++) 2 rats, sterile husk, (+) 4 rats, non-sterile husk, (**) Temperature 22-27°C, Humidity 40-70%, and Lighting 12 hours per day, (*) Temperature 10-15°C, Humidity <40%, Lighting 24 hours per day, (##) twice daily (20g/rat), (#) once daily (20g/rat).

Blood sample collection

Blood samples were collected from each animal using standard venipuncture techniques (retro-orbital sinus)

under light anesthesia (ether). Approximately 1mL of blood was collected into EDTA-coated tubes to prevent coagulation, ensuring the integrity of cellular components for hematological analysis. Sample collection procedures were performed with minimal stress to the animals.

Hematological analysis

Blood samples were processed in the Animal Hospital at Hasanuddin University. The following parameters were specifically measured and recorded are red blood cell (RBC) or erythrocyte, hemoglobin concentration (HGB), and hematocrit (HCT) count.

Statistical Analysis

All collected data were subjected to statistical analysis using IBM SPSS® 25 software. Descriptive statistics (mean pm standard deviation) were calculated for each hematological parameter across all groups. Prior to inferential analysis, data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. Given the potential for non-normal distribution and non-homogeneity, non-parametric tests, specifically the Kruskal-Wallis H test, were employed to determine significant differences among groups. Post-hoc analysis (Mann-Whitney U test) was conducted to identify specific pairwise differences when the Kruskal-Wallis test indicated significance. A P-value of <0.05 was considered statistically significant.

RESULTS

Optimizing animal models necessitates a thorough understanding of their baseline physiological state and how it is influenced by experimental conditions. Hematological parameters serve as crucial indicators of systemic health, oxygen-carrying capacity, and inflammatory status. This section presents the findings related to erythrocyte count, hemoglobin concentration, and hematocrit levels across the experimental groups, providing insights into the physiological responses of Wistar rats.

Result of hematological parameters

The mean standard deviation for erythrocyte count, hemoglobin concentration, and hematocrit levels across the various treatment groups is presented in Table 2.

Table 2: Average erythrocyte, hemoglobin, and hematocrit levels in Wistar Rats

Treatment Group	Mean of erythrocytes level ±($\times10^6$ /µL)	Mean of hemoglobin level±SD (g/dL)	Mean of hematocrit Level± SD (%)
K1	4.84±0.11	14.35±0.21	44.00±1.41
K2	4.20±0.03	12.40±0.00	38.50±0.70
K3	4.56±0.06	14.05±0.07	40.00±1.41
K4	4.75±0.02	15.35±0.07	39.50±0.70
K5	4.63±0.23	14.12±1.32	41.00±0.81
K6	4.40±0.06	12.25±0.17	42.50±1.00
K7	4.53±0.13	13.55±0.58	41.00±1.82
K8	4.42±0.01	13.40±0.40	39.50±1.00

Table 2 highlights variations in mean hematological parameters across the experimental groups. Group K1 exhibited the highest mean erythrocyte count ($4.84\pm0.11\times10^6$/L) and hematocrit (44.00±1.41%), suggesting a robust red blood cell profile. Group K4 displayed the highest mean hemoglobin concentration (15.35±0.07g/dL), indicative of strong oxygen-carrying capacity.

Conversely, K2 and K6 generally showed lower values for these parameters. The relatively low standard deviations across most groups signify consistency within the treatment groups. These baseline differences or responses to initial interventions underscore the importance of such monitoring for model validation.

Kruskal-Wallis test for erythrocyte count, hemoglobin, and hematocrit

The non-parametric Kruskal-Wallis test was applied, revealing a statistically significant difference among the groups (P=0.028). This signifies that the various experimental conditions had a detectable impact on erythrocyte levels for erythrocyte levels (Table 3). Hemoglobin data (Table 3) displayed the Kruskal-Wallis test again indicated a significant difference between groups (P=0.022), confirming that the experimental interventions significantly influenced hemoglobin concentrations. For hematocrit levels (Table 3), the Kruskal-Wallis test yielded a significant P=0.022, confirming that the treatments led to significant variations in red blood cell volume.

Table 3: Kruskal-Wallis tests for eryhtrocyte count, hemoglobin, and hematocrit

Treatment Group	Erythrocytes Count	Hemoglobin	Hemoglobin
K1			
K2			
K3			
K4	0.028*	0.022*	0.022*
K5			
K6			
K7			
K8			

Note: *(P<0.05) = Significant difference.

Visual Representation of data distribution and group differences

To further visualize the distribution and comparative differences among groups for each hematological parameter, boxplots were generated.

Fig. 1A illustrates the spread and median of erythrocyte levels. Group K1 consistently displayed higher median erythrocyte counts with relatively low variability, suggesting a stable physiological state. In contrast, other groups showed varying medians and distributions, reflecting their responses to different experimental conditions. Fig. 1B for hemoglobin indicates that Group K4 had the highest median hemoglobin levels, corroborating the mean values in Table 2, which could imply better oxygen transport capacity or specific responses to its treatment. Fig. 1C presents hematocrit levels, with K1 showing the highest median, while other groups demonstrated wider data distributions, signifying greater variability in red blood cell volume. Collectively, these boxplots visually support the statistical findings of significant differences between groups.

To identify specific pairwise differences among the groups, post-hoc analysis using the Mann-Whitney U test was performed, and the results are presented as heatmaps.

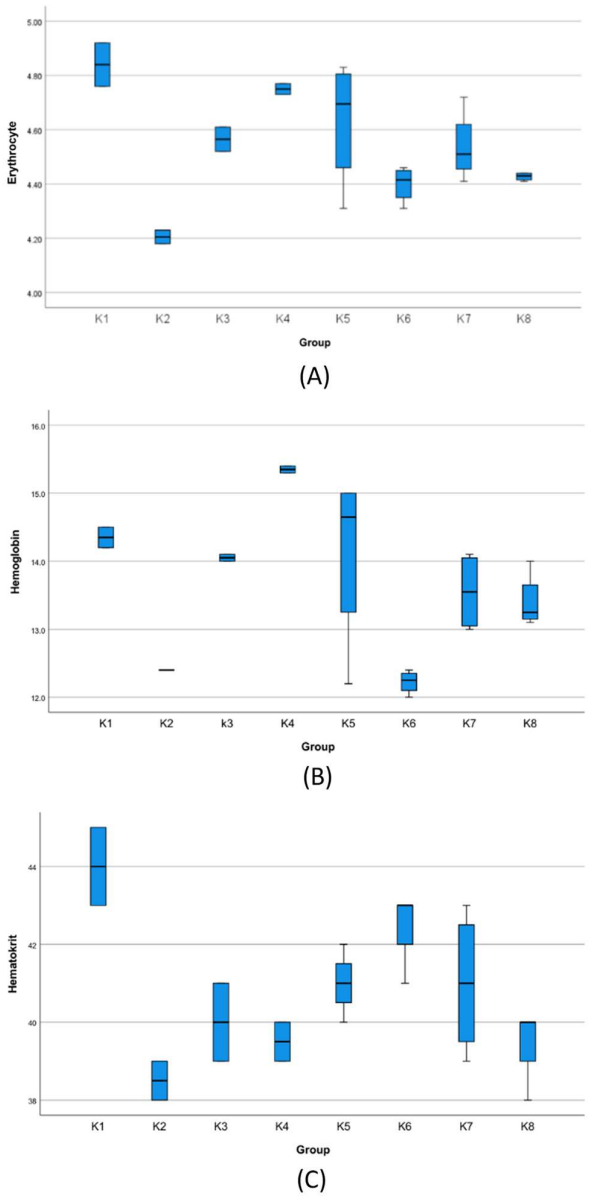

Fig. 1: Boxplots of erythrocyte (A), hemoglobin (B), and hematocrit (C) levels.

Fig. 2A, representing erythrocyte levels, shows significant pairwise differences (indicated by yellow color for P<0.05) between K1 and K2, and K1 and K8. Significant differences were also observed between K2 and K6 and between K4 and K6. The varying color combinations across the heatmap signify that not all group comparisons were statistically significant (e.g., K3 and K5 showed no significant difference, indicated by red color).

Fig. 2B for hemoglobin reveals significant comparisons predominantly involved K4 versus K2, K6, and K8, evident from the contrasting colors, which confirms that K4 maintained significantly higher hemoglobin levels compared to these groups. Some pairs, such as K3 and K5, did not show a significant difference.

Fig. 2C for hematocrit reveals significant differences between K1 and K2, as well as K1 versus K4 and K8. The color gradient from pink to dark on the heatmap indicates progressively lower P-values, highlighting stronger statistical significance. Conversely, lighter colors, such as for K5 and K7, suggest similarities (no significant difference).

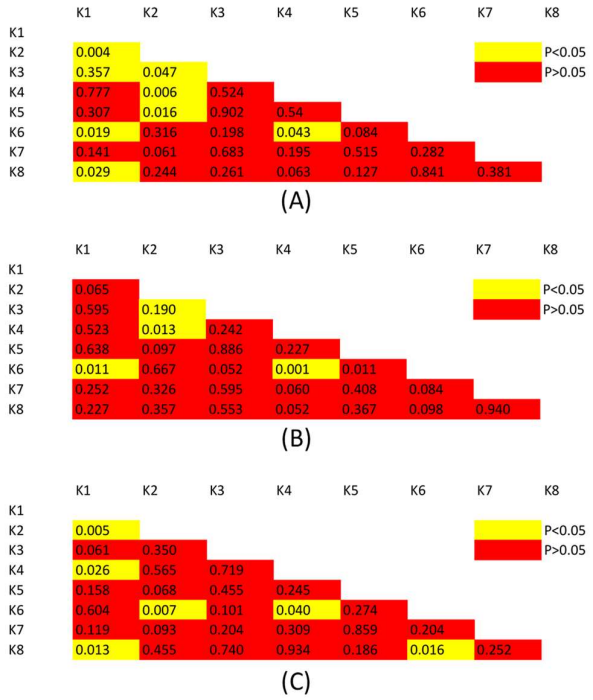

Fig. 2: Heatmap of Mann-Whitney U Test for Erythrocyte (A), Hemoglobin (B), and Hematocrit (C).

DISCUSSION

Specifically regarding hematological parameters, our findings are also consistent with more detailed research. A study by Vladislav et al. (2021) identified that several micronutrients, such as vitamin B12 and folic acid, have a strong correlation with hemoglobin levels and erythrocyte counts in rats. Although our study did not measure micronutrients, it can be assumed that nutritional differences between treatment groups may include these nutrients, which are then reflected in hematological results. Additionally, the comparison between K1 and K8, which showed significant differences in red blood cells, indicates that certain combinations of environmental and nutritional factors may have synergistic or antagonistic effects that warrant further exploration (Özturk and Çiftçi 2021; Mieske et al. 2022). Research on the combined effects of these various factors is still limited, opening opportunities for future research.

Recent studies have also revealed that iron bioavailability is significantly influenced by the expression of hepcidin and other iron-regulatory proteins, which are sensitive to inflammatory status and feeding regimes (Özturk and Çiftçi 2021; Abubakar and Tabo 2023). This might explain the lower hemoglobin levels in rats receiving inconsistent nutrition, as seen in Groups K6 and K8. Experimental studies confirm that corticosterone surges suppress red blood cell production, contributing to anemia in rodents housed under high-density or noisy environments (Mesa-Gresa et al. 2016; Onyinye and Bruno 2023).

Disruptions in gut microbiota from environmental or dietary shifts can influence nutrient absorption, especially iron and B vitamins, essential for hemoglobin production (Voelkl, 2020). This axis could offer novel insights into the systemic physiological changes observed under differing animal welfare protocols. In addition to environmental and nutritional variables, recent findings highlight the impact of oxidative stress on hematological profiles in laboratory rats. Prolonged exposure to suboptimal housing conditions increases oxidative markers, which in turn disrupt erythropoiesis and reduce hemoglobin synthesis (Abubakar and Tabo 2023).

These detailed hematological analyses provide critical insights into the physiological state of Wistar rats under different experimental conditions. Variations in erythrocyte, hemoglobin, and hematocrit levels reflect their metabolic and physiological adaptations, which are fundamental to the reliability of disease models. Understanding these parameters, especially when adhering to animal welfare principles, allows researchers to ensure that any observed outcomes are truly attributable to the disease or intervention, rather than confounding factors related to animal health or stress (Jones. 2016; Jacob et al. 2018). This optimization is key to producing robust and translatable research findings for both infectious and non-infectious diseases.

The aspect of animal welfare emphasized in this study proved to be crucial. Differences in cage conditions, including density and sanitation, can cause chronic stress in rats (Guilherme et al. 2016). Research by Adriana (2025) shows that chronic stress triggered by suboptimal housing conditions can lead to the release of corticosteroids, which ultimately affects red blood cell production, consistent with the observed fluctuations in erythrocyte values. Furthermore, research by Barros (2018) specifically links stress levels in mice with hematocrit variability, concluding that stressed animals are more likely to exhibit higher hematocrit values. Therefore, strict animal welfare protocols are not only a matter of research ethics but also a prerequisite for obtaining valid and reproducible hematology data (Bayne 2018).

These findings are consistent with several previous studies that also highlight the sensitivity of Wistar rat hematology parameters to various external factors (Grimm and Sauter 2020; Ratuski and Weary 2022). For example, a study by Abubakar and Tabo (2023) found that variations in environmental temperature can significantly alter hematocrit values, consistent with the differences observed between the groups in this study. Nutritional supplementation, as tested by Arsyad et al (2020), showed a significant increase in hemoglobin levels, which may explain the highest hemoglobin levels observed in the K4 group in this study. Conversely, an unbalanced diet or nutritional stress can lead to a decrease in erythrocyte and hemoglobin values, as documented by Grimm and Sauter (2020).

This study demonstrates that variations in environmental conditions, housing management, and nutritional regimens significantly influence the hematological profiles of Wistar rats. Specifically, differences in erythrocyte count, hemoglobin concentration, and hematocrit levels across groups reflect diverse physiological responses to experimental settings. These findings support the assertion that even moderate deviations in animal welfare practices can lead to measurable alterations in systemic function (Carratore et al. 2024).

The group with the highest erythrocyte and hematocrit values (Group K1) was housed in a low-density, clean environment with sterilized husk, suggesting that minimal stress and improved sanitary conditions may promote erythropoiesis and overall circulatory health. This is in line with previous studies indicating that improved environmental enrichment and reduced animal density can minimize stress-induced suppression of hematopoiesis (Kondashevskaia and Mkhitarov 2005; Barros et al. 2018).

Group K4, which exhibited the highest mean hemoglobin levels, was subjected to a lighting cycle of 12 hours per day, likely promoting stable circadian rhythms. Proper light–dark cycles are known to regulate melatonin and corticosterone levels, which in turn influence erythropoietin activity and hemoglobin synthesis (Voelkl et al. 2020). Conversely, Group K6 exposed to continuous lighting—showed reduced hemoglobin levels, possibly due to circadian rhythm disruption, consistent with prior findings in stress-induced anemia models (Arsyad et al. 2020; Lyudmila et al. 2021).

Nutritional factors also played a pivotal role. Group K8, fed once daily, showed significantly lower hematological indices than Group K7, which received two meals per day. Caloric restriction and inconsistent feeding patterns may lead to micronutrient deficiencies, particularly iron and B12, impairing red blood cell production and hemoglobin synthesis (Guilherme et al. 2016; Patel et al. 2024). This emphasizes the importance of controlled and adequate feeding protocols in maintaining hematological stability in laboratory animals (Benyoucef et al. 2023).

The statistically significant differences observed in all three hematological parameters reinforce the need for standardized animal welfare protocols in preclinical research (Sharma et al. 2024). Deviations in erythrocyte count, hemoglobin concentration, or hematocrit not only signal stress or physiological disruption but can also compromise the interpretation of therapeutic efficacy in disease models. In alignment with the 3Rs principle—particularly refinement—comprehensive physiological assessments help ensure animals remain in optimal condition, thus improving the reliability of biomedical findings (Kilkenny et al. 2010; Percie du Sert et al. 2020).

Taken together, these results underscore the interplay between environmental management, welfare practices, and biological outcomes in animal models. Ensuring consistency in housing conditions, lighting schedules, and feeding protocols is essential not only for ethical reasons but also for the scientific integrity of research involving hematological biomarkers.

The results of this study indicate significant variations in erythrocyte, hemoglobin, and hematocrit values in Wistar rats placed in different experimental conditions. The notable differences between the control group (K1) and several other treatment groups (K2, K4, K6, K8) indicate that factors such as cage management, environment, and nutrition have a direct impact on hematological parameters. Data analysis using the Kruskal-Wallis test and Mann-Whitney U test reinforces these findings, showing that changes in treatment conditions can modify the blood

profile of rats.

In the context of research methodology, our results support the argument put forward by Voelki et al (2020) regarding the need for standardization of experimental conditions to improve the reliability and reproducibility of research results. A study by Carratore et al. (2024) even suggests that unmanaged factors, such as noise fluctuations in the cage environment, can indirectly influence hematological data through stress mechanisms. Thus, the results of this study provide empirical evidence that a comprehensive evaluation of welfare and environmental aspects is an important step in optimizing the use of Wistar rats as a research model (Samaneka et al. 2016).

In conclusion, the results of this study reinforce the importance of standardization and strict monitoring of all aspects of animal treatment in research, from cage management and environment to nutrition. The significant variability observed in erythrocytes, hemoglobin, and hematocrit not only provides important physiological data but also serves as a warning that the validity of research may be compromised if animal welfare aspects are neglected.

Conclusion

This study effectively demonstrated the importance of comprehensive physiological and metabolic evaluation, specifically through erythrocyte, hemoglobin, and hematocrit analysis, for optimizing Wistar rat models in infectious and non-infectious disease research, while rigorously upholding animal welfare principles. Significant variations in these hematological parameters were observed across different experimental groups, indicating distinct physiological responses to various conditions or interventions. The findings underscore that a thorough understanding of an animal model's hematological profile is critical for ensuring the scientific rigor and reproducibility of research outcomes. By integrating these detailed physiological assessments with ethical animal care, researchers can enhance the validity and translational relevance of disease models, ultimately contributing to more reliable and impactful biomedical discoveries. This approach represents a significant step towards refining animal models for improved research quality and ethical practice.

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