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Diversity of blood parameters of *Oryctolagus cuniculus* var. *domestica* L. reared in different ecological regions of Zarafshan Oasis, Uzbekistan

DAVRON KHAYITOV[1,2], ZAYNITDIN RAJAMURADOV[1], BAXRITDIN BAZAROV[1,♥], MIRZOXID KUZIEV[1], MARKHAMAT ISMAYILOVA[1], NATALYA ALLANAZAROVA[1], SHARIFKUL AMINJONOV[1], OYSORA TOJIKULOVA[1], NIGORA RAKHMATOVA[1], DILNOZA NAMAZOVA[1], MUZAFFARA NORMURODOVA[1], MIRZAAHMAD KHUJABEKOV[1,2], DILOVAR KHAYDAROV[1], NURNISO RAJAMURADOVA[2]

[1]Department of Human and Animal Physiology and Biochemistry, Samarkand State University named after Sharof Rashidov. University Boulevard 15, 140104 Samarkand, Uzbekistan. Tel.: +998-66-2391305,♥email: baxritdin-bazarov@rambler.ru

[2]Vivarium (Biophysiological and Biochemical) Research Laboratory, Samarkand State University named after Sharof Rashidov. University Boulevard 15, 140104 Samarkand, Uzbekistan

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Abstract. *Khayitov D, Rajamuradov Z, Bazarov B, Kuziev M, Ismayilova M, Allanazarova N, Aminjonov S, Tojikulova O, Rakhmatova N, Namazova D, Normurodova M, Khujabekov M, Khaydarov D, Rajamuradova N. 2024. Diversity of blood parameters of* Oryctolagus cuniculus *var.* domestica *L. reared in different ecological regions of Zarafshan Oasis, Uzbekistan. Biodiversitas 25: 1465-1471.* In the last 30 years, there has been a trend of rapid pollution of the atmosphere under the influence of climate change, urbanization and anthropogenic factors. According to statistics, the quality of the products obtained from agricultural animals deteriorates under the influence of the above-mentioned stress factors in some regions of Uzbekistan, and the number of livestock decreases annually by 1.8-2.3%. In this regard, there is an increasing need to research the effects of extreme climate and abiotic factors on rabbits' physiological state and biochemical indicators to effectively maintain agricultural animals, including rabbits, in extreme conditions and increase their productivity. This study is one of the first studies of this type, aimed at determining the blood parameters of the Chinchilla breed of rabbit - *Oryctolagus cuniculus* var. *domestica* L., raised in the ecological regions of Zarafshan Oasis (Uzbekistan) with different air temperatures, rainfall, and atmospheric pollution. The lack of information in scientific sources on the variability of blood parameters in rabbits raised in different ecological regions due to changes in climate indicators and atmospheric air pollution indicates that in-depth research has not been conducted in this regard. According to the results of the research, rearing in ecological regions with different air temperatures, rainfall, and atmospheric pollution has a direct effect on the number of erythrocytes, leukocytes, and hemoglobin in the blood of rabbits, the composition of blood proteins, and indicators of cellular and humoral immunity in the blood. Changes in these indicators are observed by environmental pollution. The information presented in the article serves to explain the diversity of blood parameters in *O. cuniculus*, kept in different ecological environments, and to choose the right approach for feeding rabbits.

Keywords: Blood parameters, Chinchilla, diversity, ecological regions, metabolism, *Oryctolagus cuniculus* var. *domestica*, protein

INTRODUCTION

After 2000 years in the world, in various ecological regions under the influence of adverse factors of the external environment, there is a trend of decreasing productivity of agricultural animals and natural resistance against infectious and invasive diseases (Hayek 2022). As a result, the deterioration of the quality of the products obtained from agricultural animals and a sharp decrease in the number of heads are noted (Manyi-Loh et al. 2018; Bazarov et al. 2023).

Living organisms are self-regulating systems in response to the influence of various biotic and abiotic factors on the natural environment. Environmental variability caused by urbanization, atmospheric pollution and various extreme abiotic stress factors affects animals (Khalimov et al. 2023; Kudratov et al. 2023), plants (Nurullayeva et al. 2021; Ruziyev et al. 2023; Akhmedov et al. 2023), microorganisms (Alikulov et al. 2022; Akramov et al. 2023) affect species diversity, various physiological and biochemical parameters (Seebacher et al. 2012).

Domestic rabbits (*Oryctolagus cuniculus f. domesticus*) are important for biomedical research and agricultural animal breeding because they are mild and easy to handle, restrict, and reproduce (Zhou et al. 2018). Among rabbits, the Chinchilla breed differs from other breeds in that it can be fed both for meat and skin (Neirurerová et al. 2019). In 1910-1913, this breed was obtained by crossbreeding wild Russian mountain and air color bevern rabbits in France. The average body weight is up to 3.2 kg, body length is up to 43 cm, and chest circumference is up to 35 cm. Due to its fast-fattening properties, it is maintained in the meat direction. Also, the skin obtained from this rabbit breed is of good quality and ripe, and can be dyed in any color (Khayitov 2023). In recent years, research must evaluate the effects of extreme climate and abiotic factors on rabbits' physiological state and biochemical parameters to effectively care for rabbits in extreme conditions and increase their productivity.

The blood system reacts directly and perfectly to any negative impact of the external environment on the animal body. The fact that blood has such a feature is characterized by the fact that it is a functional system that unites many systems of the organism (Billman 2020). Therefore, changes in the blood system have constantly attracted the attention of researchers because human activities influence the environmental, physical, and chemical effects of production and economic activities. Any adverse effects in these processes are interconnected with hematopoietic cells and immune systems and are manifested in clinical immunological and hematological processes. An objective assessment of the complications of adverse factors on the condition of the blood of animals can serve as a basis for considering the dynamics of the body's adaptive mechanisms (Pietras 2017). It is important to analyze the characteristics of phenotypic adaptations of hematological deviations under the influence of adverse factors and the nature of participation of secondary functional systems.

Several scientific studies and other sources have recorded important information on the influence of external environmental factors on rabbit breeds' physiological, biochemical, and other indicators (Nakyinsige et al. 2013; Wilson et al. 2023). Also, in studies carried out in Iran and Indonesia, the effect of chemicals injected into the body of *O. cuniculus* on its meat quality indicators (Keshavarz et al. 2020), the resistance potential of bacteria isolated from the body of rabbits to pathogens was evaluated (Wulandari et al. 2022). However, in these records, the lack of data on the variability of blood parameters in rabbits raised in different ecological regions due to changes in climate indicators and atmospheric air pollution indicates that in-depth research has not been conducted in this regard.

Based on the data analyzed above, environmental factors affect the physiological and biochemical parameters of animals, including rabbits, which are kept in different conditions. In this regard, the existence of ecological regions with different air temperature, precipitation and atmospheric pollution in the Zarafshan Oasis (Uzbekistan) requires determining the effect of the conditions of these regions on animal blood parameters. Therefore, the purpose of this research work is to evaluate the changes in blood parameters in the Chinchilla breed of *O. cuniculus* raised in different ecological regions.

MATERIALS AND METHODS

Study area

Three ecological regions of Zarafshan Oasis (Uzbekistan) with different air temperatures, precipitation amounts, and atmospheric pollution were selected as research areas. When selecting the study areas, the data on air pollution, annual temperature and precipitation of the region were analyzed and distinct areas were identified. These regions are the relatively clean air region (Urgut), the air pollution region (Pastdargam), and the intense air pollution region (Samarkand). Figure 1 and Table 1 complete the description of the sampling station.

Table 1. Description of different ecological regions selected as study area in Zarafshan Oasis, Uzbekistan

Research site	Location	Coordinate	Waste materials released into the atmosphere (2022), thousand tons	Average annual temp. (2022), °C	Average annual rainfall (2022), mm
Station I (Environmentally clean area)	Bakhrin (Urgut)	39°27'31.2"N, 67°01'54.0"E	12.0	14.9	18.0
Station II (Polluted area)	Juma (Pastdargam)	39°41'42.2"N, 66°40'43.6"E	35.0	16.8	13.0
Station III (Intensively polluted area)	Samarkand (Samarkand)	39°38'52.9"N, 66°57'58.2"E	97.0	16.0	14.0

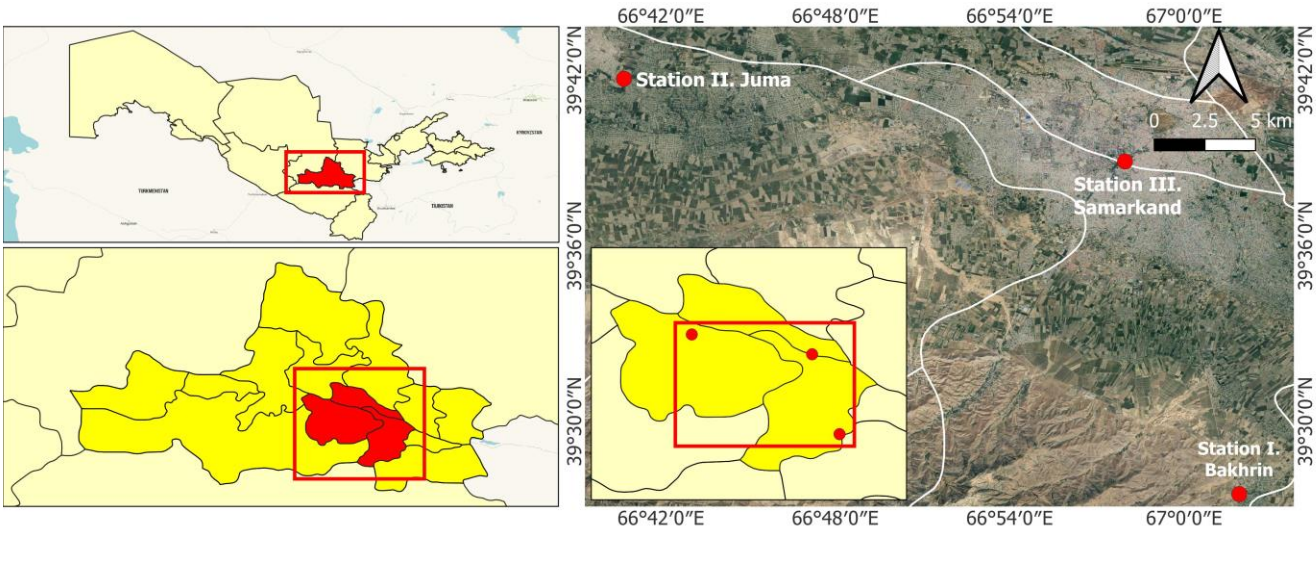

Figure 1. Research area in Zarafshan Oasis, Uzbekistan (I-Environmentally clean area, II-Polluted area, III-Intensively polluted area)

Figure 2. Research object: *Oryctolagus cuniculus* var. *domestica* L. (Chinchilla)

Animal material and experiential procedures

The Chinchilla breed of *O. cuniculus* was selected as a research object in the experiments (Figure 2). Therefore, 5 rabbits, not less than one-year-old, were attached to each group of experimental options. Data obtained at the beginning of the experiment were used as a control option for all parameters. Rabbits were reared in the selected ecological regions for 4 months (April-June 2023) and were checked for all indicators at the end of the rearing. A traditional feed ration was used in the rearing of rabbits.

Determination of the morphological composition of the blood

The method of Khayitov (2023) was used to determine morphological indicators of blood. Analysis of the number of erythrocytes, leukocytes, and hemoglobin in the blood were performed on the Automated hematology analyzer BC-5000 (Mindray). In the experiments, the blood taken from the research subjects was initially placed in vacuum tubes containing 1 mL of EDTA (Ethylenediaminetetra-acetic acid) solution. The test tube was left for 2-3 minutes. The precipitate from the test tube was taken into the device tube and placed in the analyzer. The obtained results were analyzed and average indicators were determined.

Determination metabolism of proteins in the blood

The method of Duda et al. (2020) measured the number of proteins in the blood. Analysis to determine the number of albumins and globulins (a, b, and g) in the blood were performed on the Automated clinical chemistry analyzer XL-200 (ErbaMannheium). In the experiments, the blood obtained from the research subjects was placed in a test tube of 2 mL and centrifuged at 1,500 g for 10 minutes. Next, 1 mL of the resulting supernatant was poured into the cuvette of the analyzer and placed in the analyzer. The obtained results were analyzed and average indicators were determined.

Determination of indicators of immunity in the blood

The method of Khayitov (2023) was used to determine the indicators of immunity in the blood. Analysis to determine the number of T-lymphocytes, T-helpers, T-suppressors, T-active fraction, IgA, IgM, and IgG in the blood were performed on the Fully chemiluminescence immunoassay analyzer MAGLUMI-600 device. In the experiments, the blood obtained from the research subjects was placed in a test tube of 2 mL and centrifuged at 1,500 g for 10 minutes. Next, 1 mL of the resulting supernatant was poured into the cuvette of the analyzer and placed in the analyzer. The obtained results were analyzed and average indicators were determined.

Statistical analysis

Statistical processing and drawing of the results were performed using Microsoft Excel 2013 (USA) computer program. The results of the experiment were statistically summarized by evaluating the arithmetic averages of 5 repeated experiments at the level of statistical reliability of $p \leq 0.05$. In the mathematical-statistical analysis, the mean values and deviations of the indicators, as well as the calculation of the probability, were carried out according to the method of (Lakin 1990).

RESULTS AND DISCUSSION

Morphological composition of the blood of *O. cuniculus* (Chinchilla)

Changes in blood cells, erythrocytes, leukocytes, thrombocytes, and their compositional compounds under the influence of adverse factors indicate homeostatic changes in the body, acceleration or deceleration of metabolism, and the state of immunological stagnation of the body. In our research, experiments were conducted to evaluate the effect of the ecological region in which they are kept on the morphological parameters of the blood of the rabbit breeds, which are the object of the research. According to the research results, the morphological parameters of the blood of rabbits can change depending on the climate and conditions of care (Table 2).

Table 2. The influence of the ecological region of rearing on the morphological composition of the blood of *Oryctolagus cuniculus* (Chinchilla)*

Indicators	Control (before the experiment begins)	Ecological regions		
		Station I	Station II	Station III
Erythrocytes, million/mm^3	6.9±0.5	6.4±0.6	5.6±0.3	5.2±0.4
Leukocytes, thousand/mm^3	7.1±0.3	7.0±0.4	7.3±0.2**	7.8±0.4
Hemoglobin, g/L	134.0±3.2	136.0±2.7	121.0±2.8	113.2±4.5
Color indicator of blood, CI	1.0 ±0.1	1.0 ±0.1	1.1 ±0.1	1.1 ±0.1

Note: *n = 5, ** P<0.05

Table 2 shows that the number of erythrocytes in the blood of rabbits was equal to 6.4±0.6 million/mm^3, 5.6±0.3 million/$mm^{3,}$ and 5.2±0.4 million/mm^3 in relatively clean, polluted, and intensively polluted ecological regions. Therefore, considering the number of erythrocytes in the control option is 6.9±0.5 million/mm^3, the number of erythrocytes decreased by 7.2% in rabbits raised in a relatively clean ecological region and by 18.8% and 24.6% in rabbits raised in a polluted and intensively polluted ecological region. It was found that the number of leukocytes in the blood of rabbits was equal to 7.0±0.4 thousand/mm^3 in rabbits raised in a relatively clean ecological region, 7.3±0.2 thousand/mm^3 and 7.8±0.4 thousand/mm^3 in rabbits raised in a polluted and intensively polluted ecological region. The number of hemoglobin in the blood of rabbits was equal to 136.0±2.7 g/L, 121.0±2.8 g/L, and 113.2±4.5 g/L in relatively clean, polluted, and intensively polluted ecological regions. Compared to the hemoglobin content in the blood of rabbits in the control variant (134.0±3.2 g/L), the number of hemoglobin increased by 1.5% in rabbits raised in a clean ecological region. This indicator increased by 9.7% in rabbits raised in a polluted and intensively polluted ecological region and decreased by 15.7%. It was also observed in studies that the value of the blood color indicator of rabbits does not depend on the environment in which the rabbit is kept. The fact that the blood color indicator is almost the same in all variants confirms the above.

The results of the experiments aimed at evaluating the influence of the ecological region in which they are kept on the morphological parameters of the blood of *O. cuniculus* (Chinchilla) showed that with environmental pollution, the number of erythrocytes in the blood and, depending on it, the number of hemoglobin decreases, and the number of leukocytes in the blood increases. As a result of research conducted by Chmurska-Gąsowska et al. (2021) and Skrbec et al. (2023) on different breeds of *O. cuniculus*, the number of erythrocytes in rabbits is 25-35% under the influence of climate change, urbanization, diseases and various environmental factors on the variability of morphological indicators of blood, decreased to 22-26%, and the number of leukocytes increased to 22-26%. This information confirms that the information received by us is reasonable.

During our research, we analyzed blood leukograms in rabbits raised in relatively clean, polluted, and intensively polluted ecological regions (Table 3). As a result of the experiments, it was found that the number of basophils in the blood was 5.8±0.5% in the control variant and 5.6-6.1% in the experimental variants; the highest value was recorded in the rabbits raised in the intensively polluted region. It was observed that the percentage of eosinophils in the blood of rabbits was equal to 0.9±0.1%, 1.6±0.1%, and 1.9±0.1% in relatively clean, polluted, and intensively polluted ecological regions, and these indicators increased to 35.7% from the control option. It was also noted that as pollution in the region increased, the percentage of neutrophils in the blood of rabbits increased compared to the control variant. In particular, in rabbits kept in a relatively clean ecological region, the number of neutrophils with sigmoid and rod nuclei in blood was 5.0±0.2% and 34.0±1.3, respectively. In comparison, in rabbits kept in a polluted region, this indicator was 7.0±0.2% and 43.9±1.3%. It was found that Table 3 shows that the number of lymphocytes in the blood of rabbits was equal to 36.4±1.6%, 44.9±1.8%, and 49.4±3.3% in relatively clean, polluted, and intensively polluted ecological regions. Considering that the number of lymphocytes in the control option is 42.2±3.2%, the number of erythrocytes in rabbits raised in a relatively clean ecological region decreased by 13.7%. In comparison, rabbits raised in a polluted and intensively polluted ecological region increased by 6.4% and 17.1%, respectively. In addition, it was observed that the number of monocytes in the blood of the research rabbits increased by up to 2.7 times compared to the control option in the rabbits raised in intensively polluted conditions. In general, leukogram analysis of blood in rabbits raised in relatively clean, polluted, and intensively polluted environmental regions shows that leukogram components increase following increased leukocytes with environmental pollution.

It is known that leukocytes indicate the state of the body's natural resistance factors (Adamchuk et al. 2023). In our opinion, adaptation processes in the body of rabbits undergoing a period of adaptation to new conditions are manifested, first of all, by an increase in the number of leukocytes because white blood cells, that is, leukocytes, are responsible for the implementation of protective reflexes in the body (Suckow et al. 2002).

Table 3. The influence of the ecological region of rearing on the leukogram of the blood of *Oryctolagus cuniculus* (Chinchilla)*

Indicators	Control (before the experiment begins)	Ecological regions		
		Station I	Station II	Station III
Basophils, %	5.8±0.5	5.6±0.3	5.9±0.1	6.1±0.2
Eosinophils, %	1.4±0.2	0.9±0.1	1.6±0.1	1.9±0.1
Neutrophils with rod nuclei, %	3.1±0.2	5.0±0.2	5.7±0.3	7,0±0.2
Neutrophils with segmented nuclei, %	32.6±2.3	34.0±1.3**	35.2±1.6	43.9±1.3
Lymphocytes, %	42.2±3.2	36.4±1.6	44.9±1.8	49.4±3.3
Monocytes, %	6.6±1.2	10.7±0.7	14.4±1.0	17.1±0.3

Note. *n=5; ** P<0.05

Metabolism of proteins in the blood of *O. cuniculus* (Chinchilla)

Blood proteins perform many functions in the body: they ensure the stability of oncotic pressure, pH of the blood, and the level of cations in the blood, and play an important role in the formation of immunity in complexes with carbohydrates, lipids, hormones, and other substances (Velichko et al. 2020). Albumins and fibrinogen proteins in the blood are formed in liver cells. In contrast, globulins are formed in cells of the reticular-endothelial system of the red marrow and reticuloendothelial cells of the liver.

During our research, the composition of blood proteins was studied in rabbits raised in relatively clean, polluted, and intensively polluted ecological regions (Figure 3). The number of albumins and globulins (a, b, and g) in the blood of rabbits were analyzed according to the culture environment, in the control variant, albumins were 61%, globulins were 39%. In this variant, the number of α-, β-, and γ-globulins was 12.2%, 10.9%, and 16.3%, respectively. It was noted that the number of albumin in the blood of rabbits raised in relatively clean, polluted, and intensively polluted ecological regions was 60% (40%-globulins), 56% (44%-globulins), and 52% (48%-globulins), respectively. It was also found that with the increase in the number of globulins, the number of α-, β- and γ-globulins also increased in a correct proportion to it (Figure 3). The obtained data showed that the number of albumins in the blood of rabbits decreases. The number of globulins increases with environmental pollution.

Albumins provide the main colloid osmotic pressure in the blood due to their high hydrophilicity, small size, and high concentration. It is known that if the concentration of albumins in the blood serum decreases by 30 g/L, then a change in the colloid osmotic pressure of the blood is observed. Albumins mainly serve as a plastic building material in the body. After they are formed in the liver and released into the blood, they are transported to various organs, and in each organ, they become albumins specific to this organ and one of the main cell components (Belinskaia et al. 2021).

Globulins combine with neutral salts in blood serum and are divided into two fractions -euglobulins and pseudoglobulins. The fraction of euglobulin mainly consists of γ-globulins, and the fraction of pseudoglobulins consists of α-, β- and γ-globulins. β-globulins cover several functionally important proteins. In particular, the transferrin protein is responsible for iron transport; α- and β-globulin fractions comprise lipoproteins and metal-bound proteins. The γ-globulin fraction is found in many antibodies in blood serum. If the proteins of this fraction decrease, the formation of immune cells in the body decreases, and the functional activity decreases.

Indicators of immunity in the blood of *O. cuniculus* (Chinchilla)

One of the important conditions for the efficient survival of the animal organism in the external environment is the ability to develop natural adaptive reactions (Özbey et al. 2021). Due to its adaptive mechanisms, the animal organism has the ability to cope with the force of not very high-stress effects without significant physiological function disorders. Long-term and serious stress factors, including environmental factors (heavy metals, pesticides, chlorinated organic components, etc.), lead to a violation of adaptation mechanisms, a decrease in the body's natural resistance, an increase in the body's susceptibility to various diseases, and a decrease in productivity (Wasserman et al. 2021). The activity of various immunity links undergoes constant changes under the influence of the external environment's biological, physical, and chemical factors. Therefore, our research studied cellular and humoral immunity indicators in the blood of rabbits raised in relatively clean, polluted, and intensively polluted ecological regions.

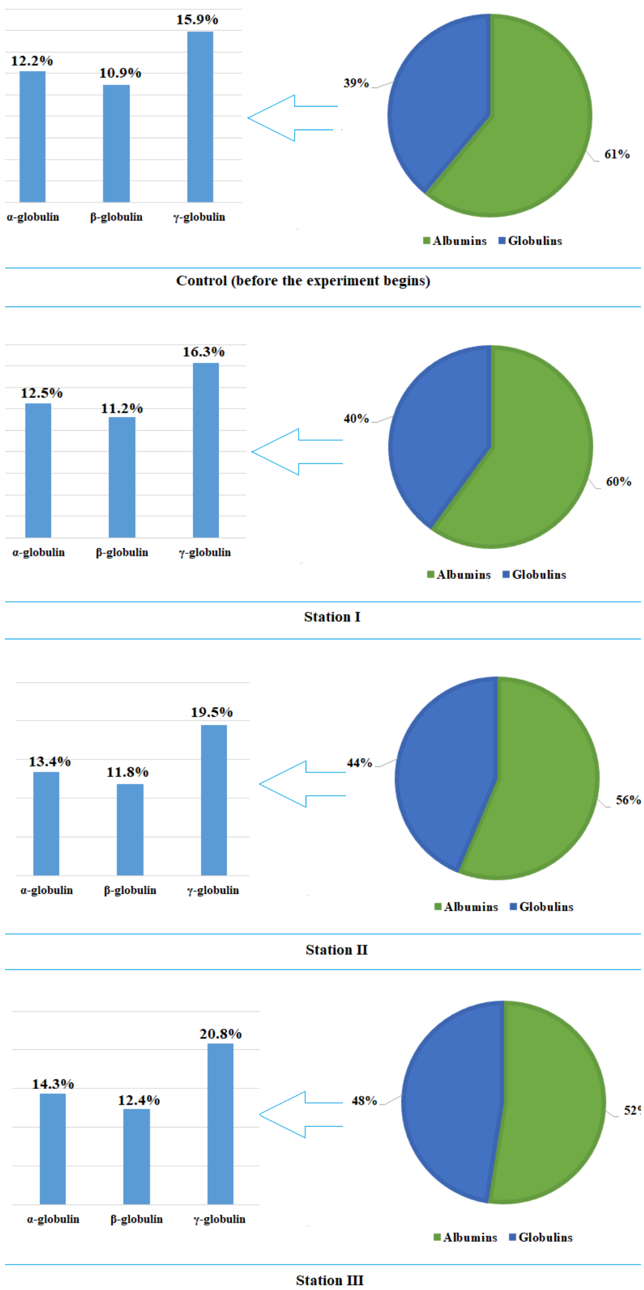

Figure 3. The influence of the ecological region of rearing on the metabolism of proteins in the blood of *O. cuniculus* (Chinchilla)

The ratio of T-lymphocytes, T-helpers, T-suppressors, T-active percentage, and T-helpers and T-suppressors in the blood determines indicators of cellular immunity in the blood of animals. Table 4 shows that the number of T-lymphocytes (3.8±0.2%) in rabbits raised in the intensively polluted ecological region increased by 0.8% compared to this indicator in the control variant (3.0±0.1%). T-lymphocyte percentage was 5.6±0.3% and 5.9±0.1% in rabbits in a relatively clean and polluted environment. With the increase in environmental pollution, there was a tendency to increase other indicators of cellular immunity (T-helpers, T-suppressors, T-activators) in the blood of rabbits. It was noted that the number of T-helpers in the blood of rabbits raised in relatively clean, polluted, and intensively polluted ecological regions was 1.4±0.1%, 1.6±0.1%, and 1.8±0.1%, respectively. The T-suppressors for these variants were 1.7±0.1%, 1.9±0.1% and 2.5±0.2%. It was noted that the ratio of T-helpers and T-suppressors decreases as the environment becomes more polluted (Table 4).

Two types of molecules differentiate foreign antigens that appear in human and animal bodies under unfavorable factors. One type is immunoglobulins, and the other is T-cell antigen receptors. Immunoglobulins, or antibodies, are glycoproteins found in all mammals' serum and tissue fluids. Plasma cells produce immunoglobulin molecules in large quantities (Chaplin 2010).

Table 4. The influence of the ecological region of rearing on the immune system cell indicators in the blood of *Oryctolagus cuniculus* (Chinchilla) (%)*

Indicators	Control (before the experiment begins)	Ecological regions		
		Station I	Station II	Station III
T-lymphocytes	3.0 ±0.1	3.1±0.1	3.3±0.2	3.8±0.2
T-helpers	1.4±0.1	1.4±0.1	1.6±0.1**	1.8±0.1
T-suppressors	1.6±0.1	1.7±0.1	1.9±0.1	2.5±0.2
T-active	1.5±0.1	1.5±0.1	1.6±0.1	1.6±0.1
Tx/Ts	0.9±0.1	0.8±0.1	0.8±0.1	0.7±0.1

Note. *n=5; ** P<0.05

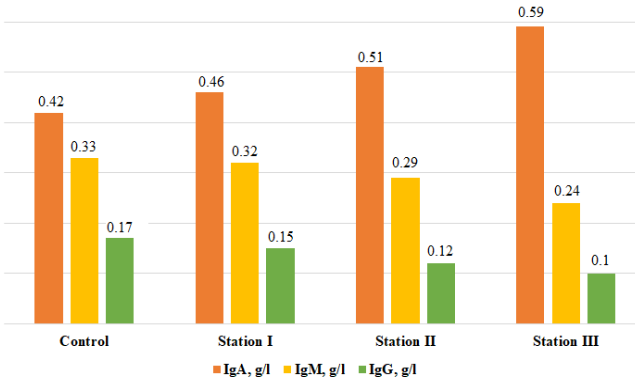

Figure 4. The influence of the ecological region of rearing on the indicators of humoral immunity in the blood of *Oryctolagus cuniculus* (Chinchilla)

Our study determined the number of IgA, IgM, and IgG as indicators of humoral immunity in blood. According to the data in Figure 4, it was noted that the number of IgA was 0.42 g/L, the number of IgM was 0.33 g/L, and the number of IgG was 0.17 g/L in the control variant. With the increase in pollution level in the ecological region, there was a tendency to increase the number of IgA in the blood of rabbits and decrease the number of IgM and IgG.

IgA makes up 10-15 percent of the total immunoglobulins in blood serum. These molecules are often found in mucous membrane secretions, such as saliva, tear and nasal fluid, skin and lung secretions, and protect its mucous membrane from the effects of microorganisms from the external environment (Pietrzak et al. 2020). IgM is the first primary immunological response. It causes agglutination and cell lysis very easily. The IgM is an antigen-detecting receptor in the V-lymphocyte membrane (Matsuda et al. 2021). IgG is found in more tissue fluids and is important in neutralizing bacterial toxins and binding to microorganisms (Palmeira et al. 2012). In addition, IgG, forming complex compounds with bacteria, activates the complement system and causes leukocytes to chemotaxis.

In conclusion, it was noted that changes in the environment affect the blood parameters of rabbits in the Chinchilla breed of *Oryctolagus cuniculus* var. *domestica* L., which were kept in ecological regions with different air temperatures, precipitation, and air pollution. The acceleration of the level of contamination in rabbits leads to a decrease in the number of erythrocytes and hemoglobin, an increase in leukocytes and their components, a decrease in the number of albumins, an increase in the number of globulins, an increase in the number of T-lymphocytes, T-helpers, T-suppressors, the percentage of T-active and T-helpers, the number of IgA increase causes a decrease in the number of IgM and IgG. The obtained data showed that the blood parameters of the *O. cuniculus* organism maintained in different ecological regions were different.

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