Leukocytes as Indicators of the Accumulation of Metals in the Body of Growing Heifers

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Abstract

The age variability of the number of heavy metals and leukocyte cells in the body of Holstein-sized Black-and-White breed heifers was studied. Blood of animals at the age of 3, 6, 9, 12, 15, and 18 months was taken, in which the leukocyte composition and the level of heavy metals were determined. It was found that the total number of leukocytes, the relative proportion of granulocytes and monocytes in the blood of heifers by 18 months of age increases by 1.34, 1.08, and 2.29 times, respectively, and the total number of lymphocytes, on the contrary, decreases by 16.74%; the amount of iron and copper increases by 31.21 and 76.47%, nickel, lead, and cadmium – 4.60, 3.50, and 2.40 times. According to the principal components method, toxic elements (nickel, lead, cadmium) are determined age variability of granulocytes, lymphocytes, and monocytes by 74.90, 77.34, and 72.70%. The research results emphasize the need for monitoring studies to develop preventive measures since the level of intake of heavy metals into the animal body is associated with the regional specifics of the territories.

Disciplinary: Animal Science, Biotechnology, Toxicology.

Cite This Article:

1 Introduction

In the industrialized regions of various countries, because of their industrialization, toxic pollutants enter and are deposited in environmental objects, which are included in trophic chains [1]. "Critical pollutants" include heavy metals, which are due to their widespread use in various industries, resistance to decay in the composition of natural environments, and the manifestation of toxic properties in living organisms, even in micro quantities [2]. In conditions of anthropogenically modified natural provinces, heavy metals are potential bioaccumulative toxicants that affect the health of animals by entering their bodies as part of feed and water [3]. It should be emphasized that some metals (trace elements) are essential, they are necessary for living

organisms to maintain vital processes. Nevertheless, if their level exceeds the threshold concentrations, they become toxic [4].

In the animal body, one of the "primary targets" of heavy metals are blood cells, which are also involved in their transport through the circulatory system [5]. It was found, that heavy metals in toxico-logically significant concentrations have immunotoxicity [6, 7, 8] due to the ability to increase the rate of generation of reactive oxygen species (ROS) that damage DNA molecules, cell membranes, and organelles [2]. According to [9], metals, as immunotoxic agents, regulate the activity and apoptosis of immune cells, the secretion of cytokines, and selective antibodies. Cytotoxic effects on monocytes, lymphocytes, and neutrophils were detected in several heavy metals [4, 10, 11]. Currently, blood leukocytes are considered sensitive targets when heavy metals demonstrate toxic properties [8, 11]. At the same time, the leukocyte blood pool and its variability allow getting an idea of the effectiveness of the processes of immunological surveillance, regulation, and protection in the animal body [12].

Although the leukocyte blood composition is associated with the ecological specialization of productive animals [4], when they are grown in agricultural enterprises located in the territories of technogenically modified natural provinces, the level of metal intake as part of the feeding diet components is not "critical" for the body in a specific age period [12, 13]. The accumulation of most elements in target organs occurs gradually and only when a "potentially toxic" level is reached, they have a negative impact on the animal immune status, the state of health, and environmental safety of the products obtained. Therefore, the determination of the tolerance level of protective forces in animal body in conditions of chronic low-dose intake of metals has not lost its relevance at present, since these processes are associated with regional characteristics of territories, specifics of the technology of animal housing, and feeding, climatic conditions, etc. [14].

In connection with the above, the purpose of this study was to determine the age dynamics of the level of heavy metals and leukocyte cells in the body of heifers of the Holsteinized Black-and-White breed during their cultivation in a natural and man-made province; to assess the effect of metals on the pool of leukocytes in heifer blood.

2 Materials and Methods

2.1 Ethical Statement

The work was carried out in accordance with the recommendations of the Bioethics Committee of the South Ural State Agrarian University (Chelyabinsk Region, Russia), considering the ethical principles of animal research.

2.2 Animals

The research part of the work was carried out in the conditions of the agricultural enterprise LLC "Unkurdinskoe" of the Nyazepetrovsky district of the Chelyabinsk region in 2021-2022.

Nyazepetrovsky district is in the mountain-forest zone of the Chelyabinsk region. Copper, iron, titanium magnetite, and apatite-titanium magnetite ores are deposited on its territory, which

determines the specifics of its industrial enterprises and the level of anthropogenic load on the environment [15]. At the same time, 35% of the district territory is occupied by agro-industrial complexes focused on the commercial production of beef and veal.

LLC "Unkurdinskoe" specializes in dairy cattle breeding and milk production. To increase milk productivity, luteinization of the Black-and-White breed is used. Young animals are grown according to the technology of dairy cattle breeding, which provides for colostrum, dairy, dairy-vegetable, and vegetable feeding periods. The feeding conditions of the animals were determined by the VIZh norms [16], the feeding ration was provided at the expense of its own feed, of which the permissible level of nickel and copper was exceeded 1.30-1.50 times as part of the grain fodder. Heifers in the colostrum and milk feeding periods were kept in individual cages, and in the milk-vegetable and vegetable periods – in groups of 15 heads each. The period of growing heifers before insemination on the farm is 18 months.

2.3 Experimental Design

To perform the work, an experimental group was formed from clinically healthy heifers (n=20) of Holsteinized Black-and-White breed (pedigree 75%) according to the principle of approximate analogs [17].

The study material was blood obtained from the jugular vein of animals in compliance with the principles of aseptic and antiseptics. Double-sided disposable needles 19G and holders of disposable vacuum systems were used for blood collection (St. Petersburg). The blood from the heifers was taken in the morning before feeding at 3, 6, 9, 12, 15, and 18 months of age, its study was conducted on the day of blood collection. Two blood tubes were taken from each head: the first for hematological analysis, the second for the determination of heavy metals. A total of 240 blood samples were taken.

Hematological studies were performed using the Mindray BC 2800 Vet hematological analyzer (China), which has species-specific settings for cattle. They included the determination of the total number of leukocytes and counting the number of leukocyte cells. The metal content was determined by the atomic absorption method using the Kvant-2A atomic absorption spectrometer (Russia) in a propane-air mixture. Blood samples were subjected to acid mineralization before analysis in accordance with GOST 26929-94 [18], the procedure for determining heavy metals corresponded to GOST 30178-96 [19] and methodological guidelines [20]. Laboratory tests were performed in the university laboratory.

2.4 Statistical Analysis

All data after checking the normality of traits distribution in the sample using the Shapiro-Wilk's test were presented in tables in the form of an average value (X) and its standard error (Sx), variability in the interval Xmin - Xmax. To assess the conjugacy of the level of the metal in the blood of heifers with the leukocyte composition of the blood, the principal components method was used [21]. The measure of traits association was determined using the Spearman's rank

correlation, the number of main components was determined by Cattell's graphical method [22]. The associations were considered statistically significant at P≤0.05. The calculations were performed in the PAST package [23]

3 Results and Discussion

As part of full blood count, which is an integral part of the assessment of the clinical status of animals [24], the leukocyte composition was determined using a hematological analyzer. At the same time, the research design provided for the study of the age dynamics of the total number of leukocytes, granulocytes, lymphocytes, and monocytes, both in absolute and relative values.

The age-related variability of leukocyte cells corresponded to the physiological norm limits. The level of total immunoreactivity of the heifer body was judged by the number of leukocytes, determining its potential ability to immune defense. The average content of cells in heifer blood during the growing period was 8.67 ± 0.26 $10.^9/l$, varying from 5.61 to 12.00 $10.^9/l$ and corresponding to the limits of the normal range (Table 1). At the same time, their number in blood samples increased with age, and at the age of 18 months exceeded the level of 3-month-old heifers by 34.62% ($P \leq 0.05$).

Table 1: Leukogram indicators of heifers (n=20), $X \pm Sx$ (X_{min}-X_{max})

Indicator	Heifer age, months						On average during the growing period
	3	6	9	12	15	18	
Leukocytes, 109/l	7.28±0.45 (5.61-9.30)	8.14±0.48 (6.20-10.50)	8.28±0.44 (7.00-10.60)	9.14±0.29* (7.90-10.40)	9.40±0.51* (7.90-12.00)	9.80±0.21* (9.20-10.80)	8.67±0.26 (5.61-12.00)
Granulocytes, 109/l	3.38±0.27 (2.30-4.60)	3.88±0.25 (3.20-5.30)	4.12±0.11 (3.70-4.60)	4.57±0.11* (4.00-5.10)	4.72±0.24* (3.90-6.00)	4.92±0.13* (4.10-5.30)	4.27±0.11 (2.30-6.00)
Granulocytes, %	46.43±1.77 (38.23-53.62)	47.67±1.00 (43.18-51.61)	49.75±3.19 (34.90-61.42)	50.00±2.09 (44.23-63.80)	50.21±1.10 (47.19-56.25)	50.20±1.00 (44.56-56.52)	49.04±0.84 (34.90-61.42)
Lymphocytes, 109/l	3.64±0.18 (2.90-4.60)	3.89±0.20 (3.30-5.00)	3.76±0.41 (2.50-6.10)	3.89±0.28 (2.70-5.40)	3.96±0.25 (3.00-5.00)	4.08±0.15 (3.10-4.60)	3.90±0.16 (2.50-6.10)
Lymphocytes, %	50.00±1.26 (41.94-57.04)	47.78±1.00 (44.31-53.22)	45.41±2.05 (35.84-56.96)	42.56±1.90* (34.17-51.92)	42.12±1.40* (35.83-48.75)	41.63±1.57* (33.69-50.00)	45.30±1.58 (33.69-56.96)
Monocytes, 109/l	0.26±0.06 (0.10-0.50)	0.37±0.08 (0.10-0.80)	0.40±0.05 (0.10-0.60)	0.68±0.10* (0.30-1.20)	0.72±0.09* (0.30-1.20)	0.80±0.10* (0.40-1.40)	0.54±0.04 (0.10-1.40)
Monocytes. %	3.57±0.54 (1.44-6.80)	4.55±0.79 (1.61-7.69)	4.84±0.56 (1.42-7.14)	7.44±0.82* (3.79-11.47)	7.67±0.90* (3.75-11.23)	8.17±1.08* (3.92-15.21)	6.04±0.16 (1.44-15.21)

Note: * - $P \leq 0.05$ relative to the age of 3 months

One of the main leukogram cells is granulocytes, represented by basophils, eosinophils, and neutrophils [25]. On average, during the growing period, the absolute count of granulocytes was 4.27 ± 0.11 $10^9/l$, fluctuating in the range X_{min}-X_{max} from2.30 to 6.00 $10^9/l$ (Table 1). At the same time, their count steadily increased in the leukocyte pool of heifer blood, and at the age of 18 months differed from the level of 3 months by 45.56% ($P \leq 0.05$). Although a similar trend was detected with respect to the relative content of granulocytes, the age increase was only 2.15%, and the average value for the study period was 49.04 ± 0.84%, varying from 34.90 to 61.42%. Definitely,

granulocytes represent a very heterogeneous population of cells, both in terms of morphology and their functions [25]. Therefore, the age variability of their total count does not allow to form an idea of the biological significance of certain cells at a certain age.

Lymphocytes play an important role in the cellular immune response of the body to the effects of exogenous factors. Their age-related variability is associated with the implementation of innate and adaptive mechanisms [26], providing, on the one hand, an increase in the absolute cell count in the blood by 12.08%, and, on the other hand, a decrease in their relative proportion in the leukocyte pool by 16.74% (Table 1). In addition to lymphocytes, monocytes participate in the implementation of an adaptive immune response, which is capable of differentiating into macrophages or ordinary dendritic cells in peripheral tissue compartments [27]. Their absolute and relative count in heifer blood increased by 3.08 and 2.29 times with age, averaging 0.54 ± 0.04 $10.^9/l$ and $6.04\pm0.16\%$ during the growing period.

In the conditions of the studied natural and man-made provinces, an important role in the formation of blood leukocyte composition is played by the level of intake into the animal body, mainly, as part of the feeding diet components, heavy metals. We have determined the age variability of those metals that predominate in the earth crust of the Nyazepetrovsky district [15]. For comparison, we used regional reference boundaries [28] established for the Chelyabinsk region and reflected the level of natural and anthropogenic impact of environmental factors on the body of farm animals.

The concentration of iron and copper was determined by metals having biological activity in an animal body and having the physiological standard boundaries (Table 2). Although the level of these trace elements in the blood of heifers increased with age by 31.21 and 76.47% ($P\leq0.05$), at the same time it was significantly lower than the regional reference boundaries. At the same time, the concentration of iron exceeded the limits of the normal range, averaging 158.20 ± 1.78 mg/l over the growing period and varying from 124.30 to 186.50 mg/l, and copper, on the contrary, was less than the minimum value of the norm interval by 1.25-2.21 times, depending on animal age. At the same time, the average copper level in the blood of heifers during the study period was 0.51 ± 0.03 mg/l, ranging from 0.23 to 0.85 mg/l.

From toxic metals in the blood of heifers of the experimental group, the age variability of lead, cadmium, and nickel levels was analyzed. At the same time, it should be emphasized that nickel belongs to "conditionally toxic" elements since its participation in the processes of erythropoietin synthesis and blood vessel growth has been proven [29]. The nickel, lead, and cadmium concentration in the blood of heifers steadily increased with age, exceeding the level of 3-month-old animals by 4.60, 3.50, and 2.40 times at the age of 18 months (Table 2). At the same time, the cadmium level was significantly lower than the regional reference limits, and nickel and lead levels, starting from the age of 9 months, fluctuated within its limits.

Thus, the animal body in the conditions of the studied natural and man-made province was most prone to the accumulation of copper and nickel, as evidenced by the change in the amount in heifer blood during the growing period.

Table 2: Metals and their age variability in heifer blood (n=20), X±Sx (Xmin-Xmax)

Metal / Reference Interval	Heifer age, months						On average during the growing period
	3	6	9	12	15	18	
Iron / 250.21-450.15	129.88±0.94 (124.30-135.20)	142.82±0.63* (140.20-146.50)	168.28±1.87* (161.00-176.00)	168.30±1.67* (163.30-177.10)	169.52±2.72* (158.80-178.20)	170.42±3.17* (158.30-186.50)	158.20±1.78 (124.30-186.50)
Copper / 0.89-1.09	0.34±0.02 (0.23-0.45)	0.44±0.01* (0.40-0.50)	0.55±0.04* (0.43-0.77)	0.57±0.04* (0.46-0.58)	0.59±0.05* (0.44-0.85)	0.60±0.03* (0.47-0.74)	0.51±0.03 (0.23-0.85)
Nickel / 0.10-0.51	0.05±0.003 (0.04-0.06)	0.08±0.01* (0.04-0.11)	0.10±0.01* (0.04-0.14)	0.15±0.02* (0.07-0.27)	0.20±0.02* (0.13-0.25)	0.23±0.02* (0.13-0.30)	0.13±0.01 (0.04-0.30)
Lead / 0.05-0.26	0.02±0.01 (0.01-0.07)	0.04±0.003* (0.03-0.06)	0.05±0.003* (0.04-0.06)	0.05±0.003* (0.04-0.06)	0.06±0.01* (0.04-0.08)	0.07±0.01* (0.03-0.09)	3.90±0.16 (0.01-0.09)
Cadmium / 0.049-0.056	0.01±0.004 (0.003-0.012)	0.01±0.005 (0.003-0.013)	0.011±0.004 (0.003-0.034)	0.013±0.001 (0.009-0.016)	0.023±0.002* (0.015-0.031)	0.024±0.006* (0.015-0.0320)	0.014±0.003 (0.003-0.034)

Note: * - $P \leq 0.05$ relative to 3 months of age; regional reference interval according to [28]

We have already noted that metals have the immunotoxicity that nickel, lead, and cadmium manifest even in conditions of their low-dose intake into the animal body [4], and in iron and copper - when the normalized limits are exceeded [2]. Although in our study it was found that the level of metals in heifer blood (iron, copper, nickel, lead, cadmium) did not exceed the regional referential boundaries, and the leukogram indicators corresponded to the normative intervals, we tried to identify elements whose age variability to one degree or another influenced the formation of the blood leukocyte composition in experimental animals groups. To this end, we used the principal components method and statistical samples "on average for the growing period". Factor loads were determined for granulocytes, lymphocytes, and monocytes. In the context of each type of cell, using Cattell's "rocky plot" graphical criterion [22], only one factor with statistically significant loads was identified – the Main component 1 (Table 3).

Table 3: Factor loads of metals on the Main component 1 in the statistical matrix of leukocyte cells

Indicators	Granulocytes, 109/l		Lymphocytes, 109/l		Monocytes, 109/l	
	Load	P	Load	P	Load	P
Iron, mg/l	0.18	0.72	-0.25	0.69	-0.33	0.62
Copper, mg/l	0.24	0.70	-0.29	0.65	0.41	0.56
Nickel, mg/l	0.78	<0.05	-0.82	<0.05	0.77	<0.05
Lead, mg/l	0.83	<0.05	-0.85	<0.05	0.81	<0.05
Cadmium, mg/l	0.80	<0.05	-0.79	<0.05	0.78	<0.05
Explained variance, %	74.90		77.34		72.70	
P	<0.05		<0.05		<0.05	

The principal componentы method showed that the bonds in metal−leukocyte cell pairs explain 74.90, 77.34, and 72.70% of the total variability of granulocytes, lymphocytes, or

monocytes, respectively, in the blood of growing heifers. Statistically significant factor loads on the pool of cells in the leukogram were detected only in toxic metals (nickel, lead, cadmium), the level of which in animal blood increased with age, contributing to their deposition in target organs as a result of low metabolic activity of the elements [2, 4]. Therefore, they influenced the proliferative activity of leukopoiesis organs, the migration activity of leukocytes, and their half-life, forming the age variability of the leukocyte blood pool in heifers

4 Discussion

In the body of growing animals, especially at the early stages of postnatal ontogenesis, the formation of immune system functions is accompanied by changes in the quantitative and qualitative composition of its cells [17. 30]. In our study, we focused on the variability of the count of leukocytes, granulocytes, lymphocytes, and monocytes. Although their variability did not go beyond the norm, it formed a certain age trend.

First, we observed an age-related increase in the total count of leukocytes by 34.62% ($P<0.05$), reflecting the immune potential of white blood cells and the presence of factors that constantly stimulate the immune system in the body of growing heifers. According to [31] data, the regulation of blood leukocyte composition is carried out by changing the phenotypes of cytokines of individual cells, determining their ability to perform their biological functions. Second, the count of granulocytes with specific cytoplasmic granules [25] increased in animal blood not so much in relative terms (by 2.15%) as in absolute terms (by 45.56%). Definitely, their total count does not allow to accurately determine the mechanism of formation of a pool of these cells, but it is logical to assume that this is associated with the manifestation of allergenic properties of metals [32, 33] in the animal body and changes in the activity of neutrophils [25], as a result of the manifestation of redox properties of some elements [2]. Third, the age variability of lymphocytes, as the main components of immune homeostasis, was accompanied by a decrease in their relative proportion in the leukocyte pool of heifer blood by 16.74%. Although these cells are prone to the effects of various endogenous and exogenous factors [34], their reactivity is directly related to factors origin, exposure intensity and duration, and body age [26]. Four, the level of monocytes in the leukocyte composition of the heifer blood increased with age, both in relative and absolute terms by 2.29 and 3.57 times ($P\leqslant0.05$). As it is known, monocytes are critical components of the body's immune defense, which is determined by their heterogeneous nature and the ability to differentiate either into macrophages or into dendritic cells. Due to this, they integrate innate and adaptive immune responses in the body [35]. This means that the systematic growth of their number reflects the demand for cells in the formation of the total immune reactivity of the animal body in the existing environmental conditions.

Consequently, the leukocyte composition of blood in the animal body reflects both the level of cellular protection and the degree of exposure to endogenous and exogenous factors, among which, in the conditions of a natural and man-made province, the level of intake of heavy metals of natural and man-made origin plays an important role.

In the body of growing heifers, the number of metals circulating in the blood increased, reaching a maximum at the age of 18 months, which was due to their presence, mainly in feed and drinking water. At the same time, the level of such elements as iron and copper did not correspond to the physiological needs of the body, determining their insufficiency in the cells of organs and tissues and the security of the associated biochemical processes [36, 37]. At the same time, the level of iron and copper in animal blood was significantly lower than the regional referential limits due to the geochemical features of the territories and the specifics of industrial enterprises [15]. A negative trend is an age-related increase in the amount of potentially toxic metals (nickel, lead, cadmium) in the body of heifers with low metabolic activity [38], the tendency to bioaccumulation [3], showing toxicity even in small amounts [29] and affecting the hematological and biochemical profile of animals [3].

Although the level of toxic elements in the blood of heifers was not "critical" when compared with regional reference norms [28], due to their tendency to bioaccumulation, they initiate the appearance of changes in almost all physiological processes. Leukopoiesis, determining the formation of blood leukocyte composition, is no exception. When assessing the relationship in a metal–leukocyte pair by the principal components method, it was revealed that the variability of the number of granulocytes, lymphocytes, and monocytes by 74.90, 77.34, and 72.70% is determined by the accumulation of nickel, lead, and cadmium in heifer body.

5 Conclusion

In the conditions of natural and man-made provinces, heavy metals are the main environmental pollutants. Their content in food chains is determined by the geochemical features of the territory and structure of industrial enterprises. In this study, using the example of the body of growing heifers, it is shown that the level of heavy metals circulating in the blood determines the direction of shifts in the blood leukocyte composition. At the same time, the total count of leukocytes, the relative proportion of granulocytes and monocytes in the leukogram of heifers by 18 months of age, compared with 3 months, increases by 1.34, 1.08, and 2.29 times, respectively, and lymphocytes, on the contrary, de-creases by 16.74%. Although the count of heavy metals in heifer blood is not "critical", according to the principal components method the toxic elements - nickel, lead, and cadmium determine the age variability of granulocytes, lymphocytes, and monocytes by 74.90, 77.34, and 72.70%.

The results of the research emphasize the regional specifics of the impact of heavy metals on the animal body; determine the need for constant biomonitoring to develop preventive measures.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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