

Cytological characteristics of blood of cows with different levels of milk productivity

Valentine Dzitsiuk*, Svitlana Kovtun

Institute of Animal Breeding and Genetics of M.V.Zubets of the National Academy of Agrarian Sciences of Ukraine, Kiev 08321, Ukraine

**Corresponding author: dzitsiuk@yandex.ua*

Abstract

Dzitsiuk, V., & Kovtun, S. (2019). Cytological characteristics of blood of cows with different levels of milk productivity, *Bulgarian Journal of Agricultural Science, 25*(Supl 1), 37–40

The article studies the content variability of erythrocytes, leukocytes, hemoglobin, ESR-index as well as numerous versions of micro-nucleus test and NOR (Nucleus Organizing Regions) Index. The studies were held for the inter-phase lymphocytes nuclei of the blood of heifers of the Ukrainian Red-and-Black breed that have various level of milk productivity. The studies displayed that the higher the productivity of heifers is the higher the erythrocyte and leukocyte values are. The direct and positive correlation between the milk production of cows and level of erythrocytes and hemoglobin is established. However, there was no connection found between the milk productivity level and the content of leukocytes in the blood. It is stated that the lowest level of erythrocytes that contain micro-nuclei is registered for the cows with yield level 3000-4000 kg. For the groups of cows that has the yield level 6000 kg and above an abrupt increase of erythrocytes with micro-nuclei was detected, comparing to the groups with the lower milk productivity. The frequencies of the lymphocytes with micro-nuclei are slightly higher for the groups that have the productivity level of 3000-4000 kg of milk, comparing to the groups of productivity of 4000-6000 kg and is lower for the groups of heifers with productivity of 6000 kg and above.

By the coloration of the samples with silver nitrate it is stated that the least NOR number in inter-phase lymphocytes nuclei is found for the heifers with milking level of 3000-4000 kg, while the highest number is noticed for the cows with the productivity level of 5000-6000 kg. The average NOR Index among all researched groups of animals is 2.36.

Key words: cows of Ukrainian Red-and-Black breed; blood; red blood cells; white blood cells; micronucleus; nucleolus organizing regions

Introduction

The blood is one of the most important internal indicators of animal organism. It has the constancy of its composition and ensures the preservation of the species, breed and individual characteristics. At the same time it is one of the most volatile and labile systems that show any changes that occur in the animal body. The properties of blood display all sorts of impacts on the organism as well. Its qualitative and quantitative composition in many ways determines the intensity of metabolism and processes of growth and development related to it.

The blood indicators that characterize the metabolism processes have a hereditary basis and relate to the productivity levels of the animals. Several research works report about indirect connection of the morphological composition of the blood with the productive qualities of farm animals of all ages. The studies of S. Batanova and O. Starostina show the correlation between oxidizing properties of blood and milk yield of cows (Batanov & Starostina, 2005). The scientific publication of E. Tkachenko explains the influence of biochemical blood parameters on the cow's milk productivity (Tkachenko, 2003).

The connection is established between the diseases of different etiology with the cytological characteristics of animal blood, including the frequencies of erythrocytes containing micro-nuclei and indices of Nucleolus organizer regions (NOR indices) in interphase cells of the animal (Loginov et al., 2004).

In order to study the instability of nuclear structures of the blood cells of dairy cows with a level of milk productivity, we studied the morphological and cytological changes in the characteristics of the animal blood with different levels of milk productivity.

Materials and Methods

The peripheral blood of heifers of Ukrainian red-motley dairy breed of “Khristinovskoe” experimental farm of State enterprise in Cherkasy region was used for the research.

The blood sampling for the cytological research was provided by taking the blood from the jugular vein into sterile syringes with heparin solution.

The number of erythrocytes and leukocytes was determined by a standard method in Goryayev counting chamber. The concentration of hemoglobin was determined according to the Sali method. The erythrocyte sedimentation rate (ESR) was calculated by Westergren method. For this purpose, the venous blood was used, previously collected with sodium citrate, 3.8% in the ratio of 4:1. The method was performed in special Westergren tubes with clearance of 2.4-2.5 mm and a scale calibrated in 200 mm. The ESR has been registered in mm for 1 hour.

For the preparation of smears for the erythrocyte micronucleus detection, the drop of peripheral blood was mixed with 10% sodium citrate solution. The smears were dried, fixed with alcohol and dyed with Giemsa stain. Under a microscopic magnification of 1000 times, the number of micronucleus polychromatic erythrocytes in 1000 cells of each sample was counted. The visible formations with a diameter of 1/5—1/20 of the size of an erythrocyte was considered as erythrocyte micronuclei. The results were registered in ppm.

The micronuclei in the leukocytes have been detected using the cell preparations, made-up after a short-term cultivation of the peripheral blood cells in vitro in a cytochalasin block using a method by Ankin et al (Sevan'kaev et al., 1995).

The nucleoli in the nuclei of lymphocytes have been found by staining the nucleolar organizers with silver nitrate (Mamaev & Mamaeva, 1992). The NOR Index was defined as the sum of nucleoli divided by the number of cells analyzed (200).

Results and Discussion

The received results of the research of the individual indicators of blood of the studied animals would reveal the level of metabolism for their organisms. The level is found physiologically normal for the researched species, according to the data taken from the analysis of the indicators of the form blood elements of erythrocyte and leukocyte chains of hemogram (Table 1).

The content of erythrocytes and their hemoglobin saturation are the important indicators that describe the level of the metabolic processes of the cows, which affects their physiological condition. For example, the difference in the level of hemoglobin content was registered at 20.2 g/l between the groups of heifers with the levels of productivity of 3000—4000 kg of milk and 7000 kg accordingly.

The erythrocyte sedimentation rate index (ESR) is the result of a very complex biological reaction, despite being methodologically very simple. For cattle, the ESR index builds up very slowly. Moreover, the main mass of erythrocytes settles after 2 hours in the interval between 2—24 hours. In our studies, no significant differences were found for the groups of heifers of various levels of productivity.

Studies have shown that the lactating cows of the Ukrainian red-motley breed, the higher the productivity is, the higher levels of erythrocytes are. The groups with the highest productivity level have the number of erythrocytes $1.5 \times 10^{12}/l$ higher than the ones with the least yield.

It is known that leukocytes enhance the mitotic activity of cells and improve tissue regeneration. The determi-

Table 1. Hematological parameters of heifers with various milk yield on the first lactation

Yield of cattle groups, kg	Number of cows	Hemoglobin, g/l	ESR, mm/h	Quantity	
				Erythrocytes, $\times 10^{12}/l$	Leucocytes, $\times 10^9/l$
3000—4000	9	101.9±0.53	1.65±0.05	6.3±0.129	8.23±0.26
4001—5000	21	115.4±0.93	1.50±0.09	7.3±0.089	8.64±0.37
5001—6000	25	118.1±1.09	1.82±0.07	7.5±0.086	9.6±0.13
6001—7000	28	118.9±0.59	1.20±0.22	7.7±0.16	9.9±0.14
7001 and more	28	122.1±0.67	1.47±0.15	7.8±0.13	9.3±0.17
Physiological norm		85—140	0.5—5.0	5.5—8.0	6.6—9.5

nation of the leukocytes in the blood of heifers of various levels of milk productivity showed the fact that the leukocyte level for the heifers of all productivity groups was within the physiological norm, however the minor intergroup difference was observed at the same time.

During the analysis of the received data we established the direct positive correlation between the milk productivity with the content of the erythrocytes in the blood. The correlation index ranged between 0.153 to 0.395. The hemoglobin level had no effect on the milk productivity as there was only a weak correlation (-0.051 to +0.240). There was no regular connection between the milk productivity and the level of leukocytes (correlation index ranged between -0.049 to +0.177).

The cytogenetic properties of animal blood that characterize the nuclear structures stability are the basis of hereditary differences in their productive qualities. The level of milk productivity is connected with the karyotypes stability or completely depends on it.

As a general rule, during the chromosomal instability determination, they study the frequencies of cytogenetic abnormalities in metaphase plates of the cultivated peripheral blood cells, which requires more time and expenses. An alternative may be the micronucleus test, which is used in two ways — for the enucleated erythrocytes and lymphocytes with an induced cytokinesis block. It was shown that the micronucleus test for the enucleated erythrocytes is not sensitively inferior to the test of studying the chromosomal aberrations in the peripheral cells of animal blood. And at the same time it is much less time consuming (Glazko et al., 2010).

The micronuclei are the nuclear structures first seen in the erythrocytes. They form in the process of division from the chromosome material that lost its contact with the spindle of the mitotic apparatus. The frequency of cells with micronuclei indicates the frequency of the cells with abnormal karyotypes and also shows the total cytogenetic instability of an organism (Il'inskih, 1992).

The studies show that the least level of erythrocytes with micronuclei is registered for the blood of heifers with a yield of 3000—4000 kg of milk. For the groups

of cows with a yield of 6000 kg and above we noted an abrupt increase in the content of erythrocytes with micronuclei to $1.7 \pm 0.03\%$ — 1.8 ± 0.21 comparing to the groups of animals with lower productivity (Table 2).

The method of the determination of the frequency of lymphocytes with micronuclei showed that the group of heifers with the lowest productivity (3000—4000 kg of milk) per 1000 lymphocytes has up to 1.10 ± 0.139 lymphocytes with micronuclei, which is little more than the group with the yield of 4000-6000 kg has and less than the group with the yield of above 6000 kg has.

The research of the nucleolar organizers of chromosomes displaying the genome activity including its part responsible for the RNA synthesis was held by cytological study of nucleoli of the inter-phase nuclei, the dimensions of which directly depend on the degree of activity of these genes (Kroker Dzhon, 1999). The nucleolar activity acts as the most revealing cytologic criteria for assessing the stability of the physiological condition of the organism. Dyeing the preparations with silver nitrate let us register the fact that the lowest number of NORs in inter-phase nuclei of the blood lymphocytes is equal to 2.09 ± 0.08 (for the heifers with 3000—4000 kg yield), the highest number was stated at 2.59 ± 0.06 (for the heifers with 5000—6000 kg yield). The average NOR index for all studied groups became 2.36.

The variability among animal groups is 0 to 18.4%. The identified quantitative changes in the number of nucleolar organizers in inter-phase nuclei of lymphocytes of the blood of heifers of Ukrainian red-motley dairy breed showed, on the one hand, the intensification of metabolic processes in immune cells, on the other hand, the possible presence of cytogenetic damage, which is confirmed by the increased number of erythrocytes with micronuclei in the blood of the cows with higher yield level.

Conclusions

Thus, the basing on all the data of the research, the following conclusions should be made. The Ukrainian red-motley dairy breed heifers with various levels of produc-

Table 2. Cytological indices of blood of cows with various yields on the first lactation

Yield of cattle groups, kg	Number of cows	Erythrocytes with micro-nuclei, ‰	Lymphocytes with micro-nuclei, ‰	NOR indices in inter-phase nuclei of blood lymphocytes
3000—4000	9	0.7 ± 0.33	1.10 ± 0.139	2.09 ± 0.08
4001—5000	21	0.9 ± 0.30	1.04 ± 0.089	2.56 ± 0.04
5001—6000	25	0.8 ± 0.23	0.98 ± 0.112	2.59 ± 0.06
6001—7000	28	1.7 ± 0.03	1.22 ± 0.090	2.29 ± 0.04
7001 more	28	1.8 ± 0.21	1.28 ± 0.083	2.29 ± 0.07

tivity have slightly different cytological and morphological characteristics of blood. This fact acts as an evidence of a particular instability of a karyotype of the animals with the higher milk productivity. Our findings support the view of researchers who believe that high-yielding cattle *ceteris paribus* has the higher levels of red blood cells compared to the less productive animals.

References

- Batanov, S. D., & Starostin, O. S.** (2005). The composition of the blood and its relationship with milk production in cows. *Zootehnika*, 10, 14-17 (Ru).
- Glazko, T. T., Stolpovskij, Ju. A., & Glazko V. I.** (2010). Genotypic and paratypic factors affecting the results of micronucleus test. *Sel'skhozjajstvennaja Biologija*, 6, 30-35 (Ru).
- Il'inskih, H. H., Novickij V. V., Vanchugova, H. H., & Il'inskih, I.N.** (1992). Mikrojadernyj analiz i citogeneticheskaja nestabil'nost', *Tomsk* (Ru).
- Kroker, Dzhon.** (1999). Areas of nucleolar organizer and fibrillar centers Rajony jadrishkovyh organizatorov i fibrilljarnye centry. *Molekuljarnaja Klinicheskaja Diagnostika. Metody*, 261-279.
- Loginov, S. I., Semenova, O. N., Iljushina, N. I., & Kulikova, S. G.** (2004). The activity of nucleolar organizer regions of chromosomes under physiological and pathological conditions in cattle. In: – *Aktual'nye Problemy Biologii, Mediciny i Jekologii*, 1, 17-19.
- Mamaev, H. H., & Mamaeva S. E.** (1992). Structure and function of nucleolar organizers (NOR): molecular, cytological and clinical aspects. *Citologija*. 34(10), 3-25 (Ru).
- Sevan'kaev, A. V., Ankina. M. A., & Zavitaeva, A. A.** (1995). A comparative study of the frequency of stable and unstable chromosome aberrations at g-irradiation of human peripheral blood lymphocytes in. *Radiobiologija*, 35, 611-617 (Ru).
- Tkachenko, E. T.** (2003) Contact biochemical parameters of blood with milk yield of cows. *Zootehnika*, 4, 17-20 (Ru).