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ORIGINAL ARTICLE

Hematological status of cows with different stress tolerance

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This study aimed to compare the hematological status of cows of different stress levels during the period of increasing milk yield (on the 60th-70th day of lactation) under the conditions of intensive maintenance technology with voluntary milking in a robotic installation. Genetic factors (breed, line) and paratypes (age, stage of lactation, type of feeding, time of year, climatic conditions) influence the hematological profile of high-yielding cows and tolerance to stress. According to the results of our research, the content of total protein and its fractions in the serum of cows' blood with different tolerances for stress levels corresponds to the recommended values, indicating the balance of metabolic processes in the body. The ratio of animal feeding was deficit-free in crude protein, as evidenced by a sufficient level of albumin in cow serum of cows (33.59-33.87 g/L) of all types of resistance to stress. Cows with a high tolerance to stress are characterized by values closer to the recommended values according to biochemical and mineral parameters of blood than animals with medium and low resistance to stress. The hematocrit content in the blood of cows with a high tolerance for stress is 1.74 and 5.08% higher than that of analogs with resistance to medium and low stress resistance, which indicates their more minor adaptation to intensive milk production technology and voluntary milking system.

Keywords: Cows, stress, hematologic status, bioessential components, microelements.

Introduction

The great practical importance in the selection of farm animals, along with exterior features, has the assessment of their interior indicators, the analysis and application of which allows not only to determine the level of adaptability, stress resistance, but also to assess productive and reproductive potential (Azab et al., 1999; Alberghina et al., 2011; Roman et al., 2020; Ruban et al., 2020; Sobolev et al., 2020; Fedorovych et al., 2021).

The complex study of cows blood composition makes it possible to judge the intensity of metabolic processes occurring in the animal's organism, its health, reproductive, and productive features (Brun-Hansen et al., 2006; Ruban et al., 2017; Bashchenko et al., 2021). Cows' blood is part of the connective tissue, which transports the body of nutrients, enzymes, vitamins, and hormones to body cells. It tends to maintain the balance of its main components, despite changing environmental conditions and changes within the body at the physiological level (Quiros-Rocha et al., 2009; Shahnawaz et al., 2011). The blood composition reflects the physiological state of the organism, which is associated with the course of vital functions and living conditions, and also determines the nature of the processes that occur in the body (Gutyj et al., 2017; Lieshchova et al., 2019; Martyshuk et al., 2019; 2020; Slivinska et al., 2021; Lieshchova & Brygadyrenko, 2021; Vlizlo et al., 2021). The dependence between blood composition and dairy cow productivity was established (Zulfiqar et al., 2012). Animals with high dairy indices are characterized by more intensive metabolic processes and higher stress sensitivity than less productive ones (Ghassemi Nejad et al., 2015; Borshch et al., 2021; Borshch et al., 2021a; Borshch et al., 2021b).

The prolonged action of various stress factors (low content of physiologically important microelements in food, climate change, or seasons) is associated with changes in the activity of stress hormones, hemopoiesis, metabolism of minerals, proteins, amino acids, carbohydrates, vitamins, lipids, free radical oxidation and antioxidant system, which is one of the fundamental molecular cell mechanisms of the pathogenesis of various pathologies (Calamari et al., 2011; Ghassemi Nejad et al., 2017; Borshch et al., 2018; Borshch et al., 2019).

The purpose of the research was to compare the hematologic status of cows of different stress levels during the period of increasing the milk yield (on the 60th to 70th day of lactation) under the condition of intensive maintenance technology with voluntary milking on a robotic installation.

Materials and Methods

The research was carried out under conditions of the robotic dairy farm conditions in the village Vylna Tarasivka, Kyiv region (49°52'28" North latitude, 30°5’12» East longitude) for Holstein heifers during the period of increasing the milk yield (1-2 month of lactation). For experiments, heifers were selected, divided by type of stress tolerance for stress into groups: high, medium, and low stress resistance.

Under voluntary stress resistance, motivational milking was determined in the robot-automatic machine was determined, where in sight of the animals from the milking stall, there was a cow in the section up to its constant leaving a cow person. Since robotic milking does not involve any human intervention, the presence of another person in a trench can become the stress factor. The beginning of milking started when putting on the first cup and the end-the moment of the last milking cup shutdown. By this type of stress tolerance, the cows were divided into three groups: high tolerance for stress-those who had no or minor conditional-reflex inhibition of milk ejection and productivity of which was not reduced; medium stress resistance in which there was up to 66.7% conditional and 33.3% unconditional-reflex inhibition of milk ejection dynamics and milk yield decreased up to 20%; low stress resistance in which more than 66.7% had conditional and more than 33.3% unconditional-reflex inhibition and a reduction of milk yield by more than 20%. The duration of milking, the productivity, and the intensity of the production were determined according to the DelProTM herd management program (DeLaval, Sweden). The dynamics of milk ejection at cows of different types of stress resistance are shown in Fig. 1.

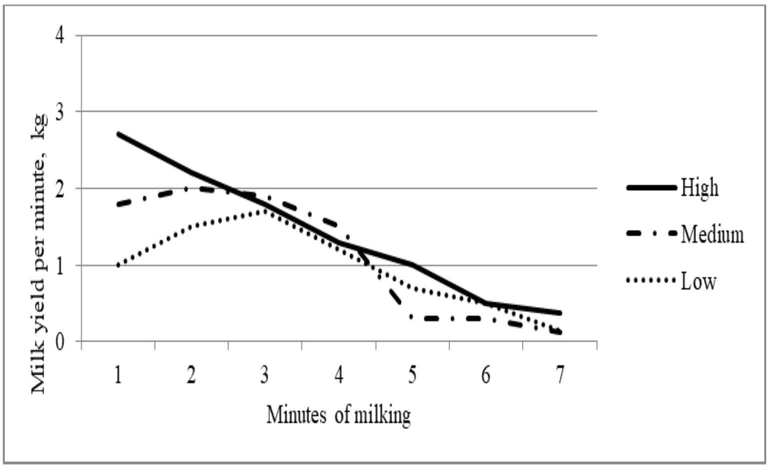

Fig. 1. Dynamics of milk ejection in cows of different types of stress resistance.

During the increase in milk yield (on the 60th-70th day of lactation), the blood of healthy experimental cows was taken before the morning distribution of the feed from the tail vein to the vacuum tubes. The biochemical parameters of the blood serum were determined on the STAT FAX 1904+ (USA) analyzer. The content of trace elements in blood was determined by the atomic absorption spectrophotometry method with the device AAS-30 (Germany). The ratio of transferees in the blood of cows was determined according to the proposed index (De Ritis et al., 1956). Analysis of the results of the biochemical studies was carried out regarding the physiological norm. The data obtained were statistically processed using STATISTICA software (Version 11.0, 2012). Student's t-test was used to estimate the statistical significance of the obtained values. Data were considered significant at $P<0.05$, $P<0.01$, $P<0.001$.

Results and Discussion

Proteins are the main and most important structural part of living organisms (van der Kuil et al., 2010; Sun et al., 2013; Li et al., 2017). A characteristic feature is the high protein lability, which, along with the feeding level, is considerably affected by the age of the animal, its productivity, physiological state, and season (Holman et al., 1934; Navis et al., 2010; Borshch et al., 2020). In our studies, the total protein content in the blood of low-stress-resistant cows dominated the established physiological norm (Table 1), indicating a more intensive physiological condition of their body compared to greater tolerance for stress cows. At cows of all types of stress resistance, albumin values were observed within the recommended norm, indicating a lack of protein deficiency in diet feeding. As a component of total protein in cows of all types of stress tolerance, the concentration of globulins exceeded the norm. The protein ratio (the ratio of albumin and globulin) indicates the state of metabolism and the intensity of protein metabolism intensity in all groups of cows. The highest content of immunoglobulins, which play a fundamental role in body humoral immunity under infectious diseases and form antiinfective immunity in newborn calves, was high tolerance for stress cows up to 21.93 g/L, that is, 3.17 and 3.83 g/L more than in animals with medium and low stress resistance. Thus, in cows with moderate and low stress resistance, their content was below the required standards, which indicates their higher sensitivity to the body's allergic and metabolic disorders.

The sublimation test shows changes in the dispersion of proteins, their size, and their ability to stay in blood plasma. Change in protein dispersion may indicate pathological liver tissue and fatty liver infiltration (Baneux et al., 2014; Sobolev et al., 2020). According to our data, this index in the cows of all groups was within the normal range, and in the cows with medium stress resistance, it was the highest. The important place in the processes of digestion and metabolism is the liver because all substances that are absorbed into the blood enter the liver, where their further transformation takes place. In the liver, ammonia is neutralized and as a result of its transformation in the ornithine cycle with the formation of urea (Higgins, 2016). Knowing the content of urea in the blood allows one to assess the supply of protein and energy and characterizes the condition of animals' ruminal digestion. The level of urea in the blood closely correlates with its content in milk. According to our research results in cows of all types of stress resistance, the urea content was within the normal range, but the animals with high tolerance for stress had slightly higher rates.

As the final product of creatine phosphate, creatinine is involved in the metabolism of proteins in the body and the energy metabolism of muscle and other tissues. Creatinine concentration in blood plasma is an essential indicator of excretory function of the body (Higgins, 2016). An excessive amount of creatinine in the blood indicates an increased consumption of high-energy or concentrated feed by an animal. At experimental cows of all groups, this indicator corresponded to normative values.

Table 1. Biochemical parameters of the blood serum of fresh cows with different stress-resistance.

Indicators	**Norm**	**Type of stress resistance**		
		High n=8	**Medium n=8**	**Low n=8**
		I group	**II group**	**III group**
Total protein, g/L	67-75	74.97 ± 0.72	73.58 ± 0.59	80.69 ± 0.63***
Albumins, g/L	30-35.5	33.59 ± 0.63	33.87 ± 0.56	33.83 ± 0.89
Globulins, g/L	30-35	41.38 ± 0.84	39.74 ± 0.92	46.86 ± 0.71***
Protein ratio, unit	0.6-1.1	0.66	0.68	0.72
Immunoglobulins, g/L	20.0-22.0	21.93 ± 0.44	18.76 ± 0.35**	18.09 ± 0.72**
Sublemate test, mL	1.60-2.60	1.76 ± 0.27	1.81 ± 0.11	1.79 ± 0.18
Urea, mmol/L	2.8-5.8	5.64 ± 0.10	5.38 ± 0.10	5.35 ± 0.13
Creatinine, μmol/L	45-140	116.30 ± 3.01	116.08 ± 5.23	117.65 ± 5.61

**P<0.01, ***P<0.001, as compared to a high-stress resistance group.

One of the functions of blood is the reflection of indicators of the physiological state of the body. Being characterized by the constant state of the chemical composition, the blood performs many vital functions in the body, in particular, nutritional, respiratory, excretory, body temperature regulation, and such as that. These functions provide a complete exchange between the organism and the environment in which the animal is located (Roland et al., 2014). Therefore, the values of hematological parameters that reflect the organism's homeostasis and represent its internal environment are based on the development of measures to prevent metabolic disorders and predict the productivity of farm animals (Kühne et al., 1989). In our studies, the level of the hematocrit index, which is the percentage ratio between the volume of blood corpuscles and plasma, was within the limits of the physiological standard in cows of high and medium stress resistance. In low-stressed cows, the hematocrit value of hematocrit was 2.80% lower than normal, which may indicate less of their adaptation to the voluntary milking system. The rate of hematocrit of cows with a high tolerance to stress was the highest and constituted 37.28%, which means that their blood contained more blood corpuscles, as evidenced by a slightly higher concentration of red blood cells in their blood, compared to their medium and low-resistant counterparts. Leukocyte content was normal, but its highest content was in the group of cows with low stress tolerance. This is because the number of leukocytes in the blood can increase during excessive physical or stressful loadings. The hemoglobin value, the main function of the transport of respiratory gases transport, was within the standard with a slight advantage in cows with high tolerance for stress–105.56 g/L by 0.98 and 3.21 g/L more than in cows with low and medium stress resistance, respectively.

The index of Hemoglobin Content in an Erythrocyte (HCE) at cows of all types of stress resistance was within the standard with a slight advantage in cows of high and medium stress. Regarding the average volume of erythrocytes, its importance in cows of all groups was lower than normal. At the same time, the lowest value was observed in cows with low-stress resistance 50.07 μm^3 or 16.55% less than the norm, in cows with medium stress resistance, this figure was lower than the norm of 14.72%, and at cows with a high stress tolerance for stress by 11.2%.

Table 2. Indicators of respiratory function of the blood of fresh cows with different stress resistance.

Indicators	Norm	Type of stress resistance		
		High n=8	Medium n=8	Low n=8
		I group	II group	III group
Hematocrit,%	35.0–45.0	37.28 ± 0.53	35.54 ± 0.71	32.20 ± 0.28***
Hemoglobin, g / L	95.0–125.0	105.56 ± 2.84	102.35 ± 3.42	104.58 ± 3.02
Erythrocytes, million / μL	5.0–7.5	6.43 ± 0.36	6.28 ± 0.47	5.73 ± 0.19
Leucocytes, thousands/μL	6.0–10.0	6.54 ± 0.41	6.57 ± 0.23	6.71 ± 0.12
HCE, pg	15.0–20.0	18.44 ± 0.34	17.06 ± 0.21**	16.24 ± 0.27***
The average volume of erythrocytes, μm^3	60.0–70.0	53.28 ± 0.77	51.17 ± 0.53*	50.07 ± 0.84*

*P<0.05, **P<0.01, ***P<0.001, as compared to the high stress resistance group.

Among the various enzymes associated with the exchange of amino acids and proteins, particular interests include Aspartate Aminotransferase (ASAT) and Alanine Aminotransferase (ALAT). These enzymes catalyze the intermolecular transfer of amino acids groups between amino and ketoacids and play an important role in the protein, lipid, and mineral metabolism of substances in animals organisms. Transamination processes occur as a result of protein-hydrocarbon metabolism. Transaminases are complex enzymes, coenzymes of which are derivatives of vitamin B_{12}. Transamination enzymes are in the liquid part of the cell and are not related to protoplasm formation; therefore, when damage to the damage of the cell's structure occurs, transaminases easily penetrate tissue fluid and blood, where they are detected (Marjani et al., 2016).

The transaminase indicators in the blood in cows of all groups exceeded the norm (Table 3). The most significant deviation from the norm was in cows of medium stress resistance, and the closest to optimal indicators were cows with low stress resistance. Cows with high tolerance for stress occupied an intermediate position.

The index of De Rhithis, or the ratio of serum transferee activity in cows of all groups, was at the proper level, with a slightly higher rate recorded in cows with low-stress resistance.

Table 3. Indicators of transaminases in the blood of cows with different stress-resistance

Indicators	Norm	Type of stress resistance		
		High n=8	Medium n=8	Low n=8
		I group	II group	III group
ASAT, un/L	10–50	64.94 ± 3.21	67.33 ± 3.86	59.90 ± 4.18
ALAT, un/L	10–40	46.87 ± 3.72	49.31 ± 3.48	41.72 ± 3.09
The index of De Rhithis, (ASAT/ALAT), un.	1.0–3.4	1.39 ± 0.26	1.37 ± 0.36	1.44 ± 0.37

As we know, calcium metabolism is closely linked to phosphorus exchange, which is required for normal protein, fat, and carbohydrate metabolism. Phosphate-calcium metabolism in the body of animals is considered to be inseparably linked to general exchange. In this case, absolute or relative sufficiency, excess, or lack of these nutritional elements can be both a reason and a consequence of changes in other metabolic processes accompanied by a disturbance of internal environment homeostasis (Dezfouli et al., 2013; Khan et al., 2015). Often, mineral deficiency causes a violation of the synthetic activity of the organic matrix of bone tissue due to changes in the quantitative and qualitative interaction relationship of organic matter of the bone tissue and the mineral component (Blanc et al., 2014). High milk productivity requires an increase in calcium intake in the diet (Hadzimusic & Krnic, 2012). In the experimental cows of all types of stress resistance, calcium deficiency was observed, while the difference between the groups was insignificant (Table 4). Analysis of this indicator showed that inorganic phosphorus content in cows of all types of stress resistance corresponded to the standard, while the highest content was typical for cows with high stress tolerance.

In the process of metabolism, closely related to calcium and phosphorus, is magnesium, which is involved in the process of energy metabolism in cells, activates several enzymes, and stimulates the production of Adenosine Triphosphate (ATP), which transports energy into cells of organs and tissues (Khan et al., 2015). Magnesium deficiency causes a slowdown in animal growth, nervous excitement, and abnormalities of muscle activity abnormalities. Cows' blood of all types of stress resistance magnesium content corresponded to the standard, although slightly fewer indicators were available for the animals of the low-stress resistance group.

The ratio of calcium to phosphorus, which indicates an adequate intake of minerals from food, corresponded to the standard in all groups of cows, but it was within the limits of minimum–1.21:1–1.24:1.

The important indicator of blood composition, which, in addition to participation in the synthesis of vitamin A, acts as a natural antioxidant, neutralizing the action of free radicals, is carotene. It increases the body's resistance to various diseases, improves reproductive function, blood formation, regulates immune responses, and enhances energy metabolism. There is a direct relationship between the content of carotene in the body of cows and their reproductive capacity. The carotene content was normal and constituted 476.37 µg/100 ml in cows with a high tolerance to stress, while these values were close to the critical limit and amounted to 457.25 and 458.54 µg/100 ml in cows with resistance to medium and low-stress resistance, respectively. Vitamin A is believed to be an irreplaceable component of the plasma membrane and acts as a receptor for signals related to differentiation and morphogenesis. Vitamin A influences respiration tissue and energy metabolism, as the rate of tricarboxylic acid oxidation depends on the vitamin content in the body (Jukola et al., 1996). In cows of all types of stress resistance, the vitamin A was within normal limits. Somewhat lower values, compared to the rest of the groups, the cows with low stress resistance had-27.19 µg/100 ml.

Table 4. Bio-essential elements, carotene and vitamin A in the blood of cows with different stress-resistance.

Indicators	Norm	Type of stress resistance		
		High n=8	**Medium n=8**	**Low n=8**
		I group	**II group**	**III group**
Calcium, mg/100 ml	2.43–3.10	2.13 ± 0.15	2.16 ± 0.09	2.11 ± 0.06
Inorganic Phosphorus, mg /100 ml	1.45–1.94	1.76 ± 0.27	1.74 ± 0.30	1.71 ± 0.23
Magnesium, mg/100 ml	0.8–1.15	0.84 ± 0.03	0.84 ± 0,01	0.82 ± 0,01
Ca:P, unit	1.2–1.6	1.21:1	1.24:1	1.23:1
Carotene, µg /100 ml	450–2000	476.37±6.72	457.25 ± 5.41	458.54 ± 7.48
Vitamin A, µg /100 ml	25–80	38.47±0.31	30.75 ± 0.62***	27.19 ± 0.56***

***P<0.001, as compared to a high-stress resistance group.

The mineral nutrition of animals is complicated by the insufficient content of moving forms of trace elements in soils and plants. Depending on the natural and climatic zone, the number of essential chemical elements is limited and their presence in separate feeds does not provide the needs of the animal's organism. The lack or excess of certain microelements in the animal diet leads to a decrease in the productivity and resistance of the organism. Their role is to act as biological activators of internal secretion processes, blood formation, cardiovascular, nervous, digestive, and sexual system functions as part of hormones, enzymes, and vitamins (Pavlata et al., 2004; Sobolev et al., 2019).

Zinc deficiency causes thymus atrophy and functional deficiency from cells of T-dependent spleen zones, lymph nodes, and lymphopenia and impaired phagocyte function. Lack of it in the body leads to reproductive dysfunction, inhibits the growth and development of young people, and brings the dysfunctions of the central nervous system and digestion processes.

The zinc content in the blood of cows was determined to be within the normal range and, to some extent, depended on the type of resistance to stress resistance (Table 5). Therefore, in cows with a high tolerance to stress, this indicator was greater than in less stress-resistant analogs and was 134.28 µg/100 ml, while in animals with medium stress resistance, it was 13.12 µg/100 ml (or 9.78%) less. Regarding cows with low stress resistance, the zinc content in their blood was in the range of the maximum permissible standard–105.38 µg/100 ml.

A cooper is a crucial microelement, a part of many enzymes, red blood cells, pigments of hair, and contained in all cells of an animal organism. The lack of copper in the rations causes anemia, growth retardation, and depigmentation (discoloration) of the hair. Domestic scientists' research has found out that the absence of sexual hunting accompanies the shortage of copper in cows. The results of our studies showed a slight advantage of the group of cows with a high tolerance for stress above the medium and low productive copper content by 1.42 and 8.12 µg/100 ml (or 1.51 and 9.50%, respectively).

Table 5. The content of microelements in the blood of fresh cows with different resistance to stress.

Indicators	Norm	Type of stress resistance		
		High n=8	**Medium n=8**	**Low n=8**
		I group	**II group**	**III group**
Zn, µg /100 ml	97.0–150.0	134.28 ± 6.87	121.16 ± 6.62	105.38 ± 5.94*
Cu, µg /100 ml	80.0–130.0	95.68 ± 3.44	94.24 ± 3.87	87.54 ± 2.37
Fe, µg /100 ml	90.0–155.0	149.56 ± 5.52	151.37 ± 5.72	149.43 ± 4.19
Mn, µg /100 ml	14.0–25.0	8.03 ± 0.36	8.17 ± 0.19	7.34 ± 0.27

*P<0.05, as compared to a high-stress resistance group.

Regarding iron content, which is an invariable element of enzymes and cell pigments, cows with medium stress resistance had a slight advantage-per 1.81 and 1.94 µg/100 ml compared to their counterparts resistant to high and low stress-resistant counterparts. In general, in all groups of animal blood, the iron content was within the range of the maximum permissible limit mark. The manganese content, which regulates several enzymatic processes in the body related to protein, fat, and carbohydrate metabolism, was lower than normal in cows with high tolerance to stress per 42.64% and in animals with medium low-stress resistance–per 41.66 and 47.58%, respectively.

Conclusion

The authors have established that the hematological profile of dairy cows depends on resistance to stress. Cows with high stress resistance, which have not undergone the conditional reflex inhibition of milk ejection and whose productivity has not decreased, are characterized by values closer to the ones recommended ones by biochemical and mineral blood indices. The authors recommend using the assessment of cow stress resistance to increase the efficiency of milk production under robotic milking conditions.

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