



## Influence of Stress on Blood Morphology

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### Abstract

Stress is a set of nonspecific changes in the body that are the result of its neurohumoral reaction to external stimuli, and its causative factors are called stressors. Changes in the body caused by a stressor are called the general adaptation syndrome, which is actually the clinical manifestation of stress. Various stresses (technological, temperature, rank ones, trauma, burns, etc.) should be considered as conditions that lead to a decrease in the level of the body's immune status and an increase in the susceptibility of animals to pathogenic and potentially pathogenic microflora that is constantly persistent in their bodies and the surrounding environment. This pattern is manifested in varying degrees in farms of different forms of ownership, in the form of stress factors that reduce the level of the immune status of the organism. The article presents the results of research on the influence of stress of regrouping on blood morphology and the immune system of newborn calves. Calves' blood was examined before and after regrouping, it was found that a decrease in the level of the immune status of the organism manifesting itself in a change in the morphological blood pattern and an increase in the susceptibility of animals to diseases, is caused by stress of regrouping, thus, stress negatively affects the overall parameters of blood morphology and calves' immunity.

**Keywords:** blood morphology, immunity, stress factor, calves, regrouping.

### 1. Introduction

H. Selye considered stress as a set of nonspecific changes in the body, which are the result of its neurohumoral reaction to external stimuli, while stressors are factors causing stress. He called changes in the body caused by a stressor the general adaptation syndrome, which is actually a clinical manifestation of stress [1]. H. Selye distinguished three stages in the development of stress or the general adaptation syndrome: 1. A stage of anxiety, which lasts several hours since the onset of a harmful factor. Changes occurring at the stage of anxiety are the symptoms that were observed by the scientist in his first experiments: these are hypertrophy of the adrenal cortex, atrophy of the thymus and lymph nodes, ulcers of the stomach and duodenum, weight loss. Following these experiments, the organism resistance to a disturbing factor at the stage of anxiety is temporarily reduced. 2. A stage of resistance or adaptation. If

the organism does not die for the first hours of exposure of a stress factor, it adapts to it over time, gets used to existing under extreme conditions (cold, noise, pain, etc.), during this period the resistance to a disturbing factor increases. 3. A stage of exhaustion occurs in case of the prolonged continuing exposure of a disturbing factor. At this stage the resistance to a harmful factor decreases again, adaptation disappears and changes, similar to those that occur in aging, occur in the organism. The main postulate of H. Selye's concept is the adaptive value of stress [2]. Various stresses (technological, temperature, rank ones, trauma, burns, etc.) should be considered as conditions that lead to a decrease in the level of the body's immune status and an increase in the susceptibility of animals to pathogenic and potentially pathogenic microflora that is constantly persistent in their bodies and the surrounding environment [3]. This pattern is manifested in varying degrees in farms of different forms of ownership in the form of stress factors that reduce the level of the body's immune status [4; 5].

The immunosuppressive effect of stress factors is related to the effect of excess of glucocorticoids and catecholamines that are formed during the development of a stress reaction of the organism. Adrenaline and norepinephrine, serotonin have immunosuppressive properties, as well as corticosteroids, which exert a depressing effect on the thymic-lymphatic system, caused by the suppression of proliferative processes and the activity of various populations of immunocompetent cells. Stress exertion can lead to a decrease in indices of nonspecific anti-infective protection, the number of polymorphonuclear leukocytes and their ability to phagocytosis, inhibition of virus-induced synthesis of interferon and interleukins. Cytokine-related interleukins, a factor of tumor necrosis play a key role in the interrelation between the immune system and the central link of the stress system [6; 7]. Being formed in response to an antigen, in immune reactions they activate the hypothalamus and the axis of the hypothalamus – pituitary – adrenal glands, including the activation of secretion of hypothalamic releasing hormones, a pituitary adrenocorticotrophic hormone and glucocorticoids. At the same time, the activity of all kinds of immunocompetent cells and the immune inflammatory reaction is suppressed. Natural killer cells are oppressed, proliferation of lymphocytes decreases, antibody synthesis is reduced [8; 9].

The change in the activity of the immune system under stress depends on 3 main factors: the force of exposure of a stressor, i.e. on the intensity of a stress response and its duration; the time of exposure of a stressor in relation to the phase of the immune response; resistance of the organism and/or its immune system to stress damage [10; 11]. In healthy animals, a mild stress response can stimulate the activity of the immune system, enhance nonspecific anti-infectious defense or cause an insignificant short-term state of decreased immunoreactivity. A prolonged stress reaction involves the suppression of the immune response, up to the development of immune deficiency [12]. Such reaction in animals develops in case of frequent movements with the purpose of forming new groups, during transportation, vaccination, diagnostic planned and unscheduled research, chipping of fangs, castration, caudoectomy, valuation, changing caregivers or their daily routine, husbandry conditions, etc.

In the epizootic process the development of a stress reaction and suppression of immunoreactivity is transient and the state of the immune system is normalized a few weeks after stress exposure. In case of longer stress stimulation, persistent immune deficiency develops, which is an important risk factor and leads to an increase in the susceptibility of the organism to infections [13; 14].

The purpose of our research was to study the impact of stress on blood morphology and calves' immunity.

## 2. Research Materials and Methods

The experiments were conducted on 29 newborn calves, which blood was examined before and after regrouping and after 10 days, the blood was taken from the jugular vein into 2 tubes, one of them was filled with a stabilizer-heparin. The stabilized blood was examined for erythrocytes and hemoglobin, leukocytes and differential white blood cell count, the phagocytic activity of neutrophils was determined by P.N. Smirnov' method [15]. In the serum, total protein was determined in a refractometric way, while albumins and immunoglobulins were determined by N.I. Blinov's method (1982) [16].

## 3. Research Results

Results of the conducted research showed that the red blood cell count in calves' blood decreased after regrouping by 14.8%, hemoglobin - by 4.2%, the total number of leukocytes decreased by 21.4%. The neutrophil phagocytic activity (NPA) decreased by 13.1%.

The number of albumins, indicating pathological changes in the liver, decreases by 25.0%. The total number of immune proteins is also reduced by 27.3% and the ratio of immunoglobulins to the total protein – by 25.7%. For all indicators characterizing the immunity, a decrease was observed after regrouping. The stress of regrouping negatively affects the overall indicators of calves' blood immunity (table 1).

**Table 1.** Calves' blood indicators before and after regrouping n=29, M±m

Indicators	Standard	Before regrouping	After regrouping
Erythrocytes, $\times 10^{12}/l$	5.0-7.5	6.2±0.2*	5.4±0.1
Hemoglobin, g/l	90-110	69.8±0.6	67.0±0.7
Leukocytes, $\times 10^9/l$	4.5-12.0	5.1±0.1*	4.8±0.1
NPA, %	30-60	31.4±0.4*	29.8±0.3
Lymphocytes, %	40-65	67.4±0.7	65.3±0.6*
Eosinophils, %	5-8	5.5±0.1	6.7±0.1
Monocytes, %	2-7	2.5±0.1	2.3±0.1
Stab neutrophils, %	2-5	3.5±0.1	3.3±0.1
Segmented neutrophils,%	20-35	23.0±0.3*	20.3±0.1
Total serum protein, g/l	50-67	60.2±0.4*	57.5±0.3
Albumins, g/l	20-38	30.5±0.4*	24.4±0.4
Immunoglobulins, g/l	20-40	19.6±0.3*	15.4±0.3
% of immunoglobulins to total protein	22-42	33.7±0.3*	26.8±0.2

Note: \* -  $p < 0.001$

## 4. Summary and Evaluation of Research Results

The conducted research has shown the influence of stress of regrouping on the immunity of an organism of youngsters. The influence of stress was manifested both on cellular and humoral factors of immunity. Humoral changes concern immune proteins, namely a decrease in the amount of total protein, albumins and globulins in blood serum. A decrease in cellular immunity factors is manifested in a decrease in the total leukocyte count and, what is especially important, the number

of neutrophils responsible for cellular defense of the organism in the form of phagocytosis decreases. The total number of lymphocytes responsible for the generation of antibodies in the body also decreases.

In general, our studies fit into the general theory of negative effects of stress on animal immunity [17]. This provision has been repeatedly confirmed by many studies of domestic and foreign authors [18]. Our experiments detailed a negative effect of stress factors on immunity at the cellular level [19]. Stress factors have a negative effect on immunity, primarily at the cellular level, which have been successfully proven [20].

Changes in the morphological blood pattern under the influence of stress factors are significant ( $p < 0.001$ ) and fit into the general knowledge of a pathological effect of stress on the organism.

## 5. Conclusion

The analysis of morbidity of calves in the prophylactic age showed that they are affected by the stress of regrouping. Research results showed that a decrease in immunity was observed for all indicators.

Thus, the conducted experiment significantly indicates the effect of regrouping on the immune system of newborn calves. A blood test performed after regrouping showed a significant decrease in all indicators as compared to blood counts before regrouping.

## References

- [1] Selye, H. (1970). *Stress without Distress*. Moscow: MEDGIZ. 336.
- [2] Selye, H. (1960). *Essays on the Adaptation Syndrome*. Moscow: MEDGIZ. 253.
- [3] Dikunina, S. S., Plavshak, L. P., Shulga, I. S., Shulga, N. N. (2015). Technological Scheme of Prophylaxis of Respiratory Diseases of Newborn Calves. *Bulletin of Krasnoyarsk State Agrarian University*. No 12. 198-202.
- [4] Shulga, N. N., Petrukhin, M. A., Zhelyabovskaya, D. A. (2012). Some Aspects of Colostral Immunity Formation in the Newborn Animals. *Bulletin of Krasnoyarsk State Agrarian University*. No 8. 136-139.
- [5] Shulga, N. N., Ryabukha, V. A., Shulga, I. S., Dikunina, S. S., Shtennikova, G. B. (2015). Pat. No. 2555140 RF, Method of Prophylaxis of Calves' Respiratory Diseases. FGBNU Dal'ZNI. Application No. 2014105001/13.
- [6] Shulga, N. N. (2015). Pat. No 2538128 RF. Method for Assessing Stress Sensitivity of Newborn Calves. FGBNU Dal'ZNI. - Application No. 2013121160.
- [7] Kireev, I. V. (2017). Dynamics of Immunoglobulins in the Organism of Cattle in the Conditions of Development of Oxidative Stress. *Vetkorm*. No 2. 42-44.
- [8] Bagchi, D., Sen, C. K., Ray, S. D., Das, D. K., Bagchi, M., Preuss, H. G., & Vinson, J. A. (2003). Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 523, 87-97.
- [9] Dhabhar, F. S. (2002). A hassle a day may keep the doctor away: stress and the augmentation of immune function. *Integrative and Comparative Biology*, 42(3), 556-564.
- [10] Bishop, C. R., Boggs, D. R., Warner, H. R., Cartwright, G. E., & Wintrobe, M. M. (1968). Leukokinetic studies: XIII. A non-steady-state kinetic evaluation of the mechanism of cortisone-induced granulocytosis. *The Journal of clinical investigation*, 47(2), 249-260.
- [11] Maxwell, M. H. (1993). Avian blood leucocyte responses to stress. *World's Poultry Science Journal*, 49(1), 34-43.
- [12] Al-Murrani, W. K., Kassab, A., Al-Sam, H. Z., & Al-Athari, A. M. K. (1997). Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. *British Poultry Science*, 38(2), 159-163.
- [13] Campo, J. L., & Davila, S. G. (2002). Estimation of heritability for heterophil: lymphocyte ratio in chickens by restricted maximum likelihood. Effects of age, sex, and crossing. *Poultry science*, 81(10), 1448-1453.
- [14] Al-Murrani, W. K., Al-Rawi, A. J., Al-Hadithi, M. F., & Al-Tikriti, B. (2006). Association between heterophil/lymphocyte ratio, a marker of 'resistance' to stress, and some production and fitness traits in chickens. *British Poultry Science*, 47(4), 443-448.
- [15] Smirnov, P. N. (1989). Assessment of natural resistance of the organism of agricultural animals. - Novosibirsk. 20.
- [16] Blinov, N. I. (1982). Methodical recommendations for determining non-specific resistance in newborn calves. Moscow: MBA.
- [17] Voloshchuk, P., Dudin, V. (1980). Reduction of the effect of stress factors on weaning of mice. *Pig breeding*. No 6. 19-21.
- [18] Kravchenko, N. (1980). Stress and its prevention in poultry farming. *Poultry farming*. No 4. 22-24.
- [19] Mogilenko, A. F. (1981). Seduxen in the prevention of stress in young cattle. *Collection of research papers*. Leningrad Veterinary Institute, 66: 53-68.
- [20] Romanyuk, B. P., Stoyanovsky, S. V. (1982). Transport stress and quality of meat. *Veterinary Medicine*. No 6. 60-61.