Prevention of transport stress in imported heifers improves their health status and their productive parameters

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Abstract. The dynamics of bioamines in platelets, neutrophils, lymphocytes and blood plasma of imported heifers indicated that experimental group of animals experience stress, which is accompanied by an adequate release of bioamines from the deposit sites: catecholamines - by 9.2-17.1%, histamine - by 2.2-6.1% and serotonin by 1.0-3.9% (P <0.05-0.001). Intramuscular injection of PS-7 and Prevention-N-C preparations to transported animals had a supportive effect on the formation of body biochemical adaptation mechanisms to the conditions of long-term transportation. After application of biopreparations the improvement of the reproductive traits of heifers of the 1st and 2nd experimental group was indicated the age of successful insemination was shortened by 1.35 and 1.53 months, the first calving age - by 1.0 and 1.2 months, the insemination index - by 1.42 and 1.93 times, the service period - by 8.8 and 12.2 days and increased fertilization after the first insemination by 1.7 and 2.0 times (P <0.05-0.01), rather than in the control. Heifers of the 1st and 2nd experimental group surpassed the peers of the control group in milk yield for 305 days of lactation by 109 and 125 kg (P <0.05), fat content by 0.16 and 0.27% and protein content by 0.04 and 0.07%.

1. Introduction

Intensification of dairy cattle breeding has increased interbreed competition, a fact that has led to an expansion of the breeding area and an increase in the number of animals of the most highly productive breeds of the world gene pool, and particularly Holstein breed [1, 2].

One of the stressors that livestock animals receive is owing to transport. Transport of livestock may expose animals to physical stimuli such as altered external conditions or changes in temperature, physiological stimuli such as restriction of feed and water during transport, and psychological stimuli due to exposure to new environments [3]. Changes in the environment during transport stimulate emotions and lead to physical fatigue, resulting in physiological changes in livestock. Thus, transport is considered to be a cause of discomfort and stress in livestock [4].

During the transportation of animals, physical, mental and vestibular loads lead to significant shifts in many physiological processes in the body. Stress factors not only affect the metabolic profile but also complicate the process of childbirth and the postnatal period is also disrupted. The risk of developing pathological conditions increases, the most pronounced of which is transport fever, ending in the death of an animal [5-7].
In addition, the lack of post-transit facilities and inadequate feed and water supply can lead to post-transport stress [8]. These stresses have negative consequences for the health of livestock such as changes in biochemical function, endocrine balance, and pathological variables, and can sometimes even lead to death [9].

While research has been carried out on the physiological and behavioral changes in ruminant livestock owing to stress after transport, most of these studies have been carried out on young calves and cattle. In particular, there are few studies on the changes in physiology and productivity in milking cattle. Transport stress increased blood parameters including leucocyte, neutrophil, and monocyte numbers by increased cortisol levels, but did not affect erythrocytes, hemoglobin and hematocrit levels. Additionally, transport resulted in a decrease in milk yield and reduced milk quality owing to an increase in milk SCC [10].

Cull dairy cows are vulnerable to transport stress even in journeys shorter than 8 h [11]. Long-term effect of transport stress in pregnant cattle causes negative response of not only female adults, but also the fetuses. By negative, one should understand abortions (7%), stillbirth (9.3%) and birth of hypotrophic baby calves (10.3%). With abortions and stillbirths (13.3%), only 86.7% of calves were born alive. The growth pattern of newly born calves was found to be linked to the effect of transport duration on their mothers, which causes the adults to give birth to lightweight calves, and baby calves to gain less in average daily weight [12].

In the light of the above, the search for multifaceted biopreparations for preventing the negative impact of stress factors on imported heifers, enhancing the organism’s adaptation to new environmental conditions and realizing the bioresources potential in affecting the reproductive and productive traits of heifers is an urgent problem of modern veterinary science and practice [13-15].

The purpose of this work is the prevention of transport stress and the realization of the bio-resource potential in improving the reproductive and productive parameters of imported heifers by activating the nonspecific resistance of the organism with PS-7 and Prevention-N-C biological preparations.

2. Materials and methods

The experimental part of the research work was carried out at the dairy-commodity complex of Agrofirma Myascom LLC, Lyskovsky District, Nizhny Novgorod Region, and the materials were processed in the Lyskovolinterdistrict Veterinary Laboratory of the State Budgetary Educational Institution “Gosvetupravlenie (State veterinary authority) of Lyskovsky District of the Nizhny Novgorod Region” and in the laboratory of the Department of Morphology, Obstetrics and Therapy FSBEI HE Chuvash State Agricultural Academy in the period from 2015 to 2019.

The animals of the research were Holstein heifers imported from the USA. The animals were transported by ferry for 3 weeks and then by cattle carriers for 2 days. In the experiment, three groups of 10 heifers each were formed according to the principle of analogs, taking into account the clinical and physiological state, age and body weight.

In order to prevent transport stress in heifers and improve their reproductive and productive traits, biological products developed by scientists of the FSBEI HE Chuvash State Agricultural Academy were used: PS-7 and Prevention-N-C. The heels of the 1st test group were injected intramuscularly with PS-7 at a dose of 10 ml twice 7 days before transportation and 2 days after delivery, the 2nd test group received Prevention-N-C at the same dose and time by intramuscular injection, and the control group did not receive anything. The preparations were aqueous suspensions containing a saccharomyces cerevisiae polysaccharide complex immobilized in an agar gel with the addition of a benzimidazole derivative and bactericidal preparations from the groups of penicillins and cephalosporins, respectively.

The concentration of bioamines (catecholamines, serotonin, histamine) in platelets, neutrophils, lymphocytes and blood plasma was set on a LYUMAM-I2 luminescent microscope (Russia) with a set of light filters adequate to the fluorescence regime of bioamines. Cytospectrofluorimetry was performed with a photometric nozzle FMEL-1A a 1.5 probe for blood structures with a serotonin interference filter 8 (525 nm), a histamine filter 7 (517 nm) and a catecholamine filter 6 (480 nm).
Photocurrent recording was performed in conventional units of recorder scale.

Number of erythrocytes, concentration of hemoglobin, total number of leukocytes and their types were determined on the PCE 90 Vet automatic veterinary hematology analyzer (Erma Inc, Japan). The status of the instrument, measurement, and plotting are displayed on a large LCD display. The device is controlled using an integrated compact keyboard. The analyzer automatically takes the blood sample, dilutes it, mixes it, lyses it, supplies it and washes it.

The total protein level and protein spectrum in serum were determined on the IDEXX VetTest 8008 biochemical analyzer (IDEXX, Russia). The VetTest analyzer offers to perform a series of steps, accompanying each of its offerings with a short audio signal, which helps the user prepare the pipette dispenser in time, insert a sample, and begin the analysis. The dispenser automatically takes the required amount of sample and then distributes it to the slide in sequence of 10 μl. As the sample passes through the slide layers, biochemical reactions occur that result in successive color changes. The VetTest analyzer optical system determines colors and their intensity. The analyzer converts the measured results into numerical measure values that are displayed on the analyzer screen and printed.

Then, phagocytic activity of leukocytes was determined using the daily agar culture of Staphylococcus aureus, lysozyme activity of blood plasma – by agar culture Micrococcus lysodeikticus, bactericidal activity of blood serum – using daily agar culture of Escherichia coli, as well as the amount of antibodies in the serum was measured on a photoelectrocolorimeter FEK-56M (Zagorsky Optical and Mechanical Plant, Russia).

Physical and chemical indicators of milk were investigated according to the protocols of state standards: solid - in accordance with GOST 3626-73, the dry fat-free dairy rest - GOST P 54668-2011, a mass fraction of fat - GOST 5867-90, a mass fraction of protein - GOST 25179-2014, lactose - GOST P 51259-99, mineral substances - GOST P 55331-2012, density - GOST P 54758-2011, acidity - in accordance with GOST P 54669-2011.

The main results of the studies were processed by the method of variation statistics on the reliability of difference of compared indicators (P < 0.05-0.001) using the Microsoft Excel software complex.

The dairy-commodity complex adopted a stall-pasture system and a loose-box method of keeping heifers. The microclimate in the barn and maternity ward corresponds to zoo-hygienic standards. Balanced feeding of heifers with the use of complete feed mixtures was performed by taking into account the physiological state of the organism and the productivity of animals. Diet included: corn silage, haylage mixed grass, concentrated feed, premixes and energy components. Animal diets provide the body with energy and nutrients, minerals and vitamins according to feeding standards.

3. Research results

Bioamines play an important role in the adaptation of the body to extreme conditions. At the same time, their ratio in the blood not only reflects but also determines the state of the vegetative-humoral-hormonal system.

Catecholamines participate in the regulation of all types of metabolism, increase the transfer of electrolytes through membranes, the absorption of O2 by tissues, improve blood supply and stimulate the work of skeletal and cardiac muscles, cause an increase in blood pressure in the vessels of the digestive apparatus, have an exciting effect on the central nervous system, increase heat formation and basic metabolism.

Serotonin is involved in the regulation of nervous, cardiovascular, digestive and immune systems, as well as having protective significance in stress. Serotonin acts as a mediator, changing the excitability and sensitivity of the nervous system. The effect of serotonin on the organs of the digestive system is to stimulate the secretory activity of the glands, for example, to increase the production of enzymes of gastric, pancreatic and intestinal juice, mucus; Enhancement of stomach and small intestine motility.

The physiological role of histamine is reduced to three main effects: reduction of smooth muscle of
organisms, expansion of microvasculature and stimulation of secretion of some glands. Histamine increases the resistance of the body.

The concentration of catecholamines in the blood of heifers is presented in Table 1.

**Table 1.** Effects of PS-7 and Prevention-N-C biopreparations on catecholamines levels, std. unit.

| Group of animals | Catecholamines in blood components | before transportation | after transportation |
|------------------|-----------------------------------|-----------------------|---------------------|
|                  | platelets                         | neutrophils           | lymphocytes         | plasma              |
| 1 experimental   | 132.8±1.31                        | 132.7±1.16            | 131.3±1.03          | 124.9±0.99          |
| 2 experimental   | 129.8±1.29                        | 127.3±1.02            | 130.4±0.98          | 122.5±1.08          |
| Control          | 130.3±3.01                        | 128.5±1.29            | 131.2±0.73          | 120.5±0.71          |

Notes: Differences between each experimental group and controls: ** P<0.01; *** P<0.001.

The established changes in the blood of heifers of the control group after their long-term transportation, namely an increase in the concentration of catecholamines in platelets from 130.3±3.01 to 152.6±2.63, in neutrophils from 128.5±1.29 to 147.0±2.27, in lymphocytes from 131.2±0.73 to 143.3±1.98 and in plasma from 120.5±0.71 to 135.4±2.76 indicate the manifestation of stress reactions. It should be noted that the concentration of catecholamines in the blood components of the heifers of the experimental groups was lower by 7.1 – 18.9% (P<0.01-0.001) compared to the controls. This decrease could be possibly attributed to PS-7 and Prevention-N-C that mobilized the sympathoadrenal system and contributed to the adaptation of the body to the pressure of the transport stress.

Serotonin levels in the blood of heifers are presented in Table 2.

**Table 2.** Effects of PS-7 and Prevention-N-C biopreparations on serotonin levels, std. units.

| Group of animals | Serotonin in blood components | before transportation | after transportation |
|------------------|-------------------------------|-----------------------|---------------------|
|                  | platelets                     | neutrophils           | lymphocytes         | plasma              |
| 1 experimental   | 309.1±2.47                    | 305.3±2.68            | 321.7±2.51          | 301.5±1.73          |
| 2 experimental   | 302.9±3.31                    | 307.8±3.46            | 323.8±3.68          | 295.7±1.27          |
| Control          | 303.6±2.32                    | 307.7±2.13            | 317.1±2.41          | 294.5±2.09          |

Notes: Differences between each experimental group and controls: ** P<0.05; *** P<0.01.

From the data shown in Table 2, it can be concluded that the intramuscular injection with PS-7 or Prevention-N-C increased the serotonin content by 2.2 – 4.1%, and 2.7 – 5.7% (P<0.05-0.01), compared with the control, respectively. Consequently, biopreparations cause activation of the serotoninergic system and, as a consequence, of adaptive processes to long-term transportation. The increased competitiveness of serotonin in relation to catecholamines, which was most characteristic at the end of the experiment, indicates the stabilization of the stress reaction, which is confirmed by the relative balance in the functional activity of the sympathetic-adrenal and serotoninergic systems.

Histamine concentration in the blood of heifers is presented in Table 3.
The changes of histamine levels in platelets, neutrophils, lymphocytes and blood plasma was similar to that of catecholamines. Biopreparations possibly activated the function of the histaminergic system involved in the processes of adaptation of the animal organism to transportation conditions.

Heifers of the control group at the 1st day after transportation showed an increase in body temperature by 0.84 °C, pulse rate – by 5.9 oscillations/min and breathing – 5.9 dv/min (P<0.01), the number of red blood cells – at 1.60×10^{12}/l, hemoglobin – at 21.2 g/l and leukocytes – at 10.71×10^{9}/l (P<0.001). In the leukocyte formula, eosinopenia, lymphopenia, and neutrophilosis with a shift of the nucleus to the left were noted. A decrease in total protein concentration by 13.3%, albumin - by 26.8% and gamma globulins - by 8.2% (P <0.01-0.001) was found. Transportation of animals causes a decrease in phagocytic, lysozyme and bactericidal activity of the blood and the level of immunoglobulins and, conversely, an increase in the activity of transamination enzymes (P <0.001). This picture of the physiological state characterizes the manifestation of a strong stress on the body.

The use of biopreparations PS-7 and Prevention-N-C has a supportive effect on the adaptation of imported heifers to the conditions of carriage, by mitigating or preventing the negative effects of stressors on the physiological status. Changes in the morphological composition of blood under the influence of intramuscular injection of biopreparations can be characterized as an increase in the protective-adaptive responses of animals to the action of transport stress. As indicated, the number of erythrocytes in the blood of heifers of the experimental groups was lower compared to the control by 14.0 and 14.2% (P <0.001), that of hemoglobin by 12.9 and 11.7% (P <0.01), leukocytes by 58.2 and 52.1% (P <0.001), band neutrophils by 5.5 and 6.4% and segmented neutrophils by 12.1 and 11.6% (P<0.001); monocytes, on the contrary, had higher values by 0.29 and 0.33% (P > 0.05), and an increase was also observed for eosinophils by 1.6 and 2.1 times and lymphocytes by 16.7 and 16.5% (P <0.001).

The use of biologics ameliorates the negative changes in protein metabolism, with a slight decrease in the level of total protein and an increase in its globulin fraction, especially gamma globulins, by 13.8 and 17.9% (P <0.001).

The dynamics of the main indicators of nonspecific resistance of the organism of the heifers are clearly presented in figures 1-4.

### Table 3. Effects of PS-7 and Prevention-N-C biopreparations on histamine levels, stnd.units.

| Group of animals | platelets | neutrophils | lymphocytes | plasma |
|------------------|-----------|-------------|-------------|--------|
| **before transportation** | **after transportation** |
| 1 experimental | 461.7±2.34 | 436.2±2.71 | 431.3±3.13 | 361.5±3.16 |
| 2 experimental | 459.2±2.74 | 442.1±2.33 | 429.2±3.09 | 359.4±2.71 |
| Control | 460.7±3.51 | 436.4±3.77 | 429.1±2.23 | 368.3±2.68 |

Notes: Differences between each experimental group and controls: * P<0.05; ** P<0.01; *** P<0.001.
Figure 1. Dynamics of bactericidal activity.

Figure 2. Dynamics of phagocytic activity.

Figure 3. Dynamics of lysozyme activity.

Figure 4. Dynamics of the concentration of immunoglobulins.

The use of biologics minimized the effect of stressors on cellular and humoral factors of nonspecific resistance of the organism in animals of experimental groups. Within 10 days, indicators
of nonspecific resistance were restored and the duration of adaptation to the effects of adverse factors during the transportation was reduced.

Indicators of reproductive traits of heifers are shown in table 4.

Table 4. Effects of PS-7 and Prevention-N-C biopreparations on reproductive qualities of heifers.

| Indicator                                      | Control | 1 experimental | 2 experimental |
|------------------------------------------------|---------|----------------|----------------|
| Number of animals                              | 10      | 10             | 10             |
| Age of fruitful insemination, months.          | 19.45±0.41 | 18.1±0.35 | 17.92±0.30 |
| Live weight of heifers at the first insemination, kg | 396.5±9.10 | 390.3±8.40 | 386.7±8.70 |
| The duration of pregnancy, days               | 285.4±2.01 | 289.1±2.13 | 287.4±2.17 |
| Age of first calving, months.                  | 28.7±0.37 | 27.7±0.32 | 27.5±0.33 |
| Insemination index                            | 2.7±0.44 | 1.9±0.27* | 1.4±0.21** |
| Service-period, day                            | 111.3±3.96 | 102.5±3.12 | 99.1±2.98 |
| Fertilized Chicks:                             |         |                |                |
| the first heat                                 | 3       | 5              | 6              |
| the second heat                                 | 4       | 4              | 4              |
| the third heat                                  | 3       | 1              | -              |

Notes: Differences between each experimental group and controls: * P<0.05; ** P<0.01.

According to the data of table 4, the lowest age of successful insemination was observed at the heifers of the 2nd experimental group (17.92±0.30 months), which turned out to be lower by 0.18 and 1.53 months than in the heifers of the 1st (18.1±0.35 months) and the control (19.45±0.41 months) experimental group. The live weight of the heifers of the control group at the first insemination was 396.5±9.1 kg and was higher than in animals of the 1st (390.3±8.40 kg) and 2nd (386.7±8.70 kg) experimental groups by 6.2 and 9.8 kg, respectively.

The maximum age of the first calving was in animals of the control group and averaged 28.7±0.37 months, exceeding the corresponding data in animals of the 1st and 2nd experimental groups by 1.0 and 1.2 months, respectively. The index of insemination of cows of the 1st and 2nd experimental groups (1.9±0.27 and 1.4±0.21) was lower by 1.42 (P<0.05) and 1.93 (P<0.01) times, respectively, than that in animals of the control group (2.7±0.44). The service period for heifers of the 1st experimental group (102.5±3.12 days) was shorter by 8.8 days (P<0.01), and of the 2nd (99.1±2.98) by 12.2 days (P<0.01), compared to that of the controls (111.3±3.96 days). It was found that in the control group, 30% of cows were fertilized in their 1st heat; this percentage was 50% in the 1st experimental group and 60% in the 2nd experimental group.

Thus, intramuscular injection with the biological preparations PS-7 and Prevention-N-C stimulates the reproductive function of heifers.

Indicators of productive performance of heifers are presented in table 5.

The highest milk yield (for 305 days during the first lactation) was observed in the heifers of the 1st and 2nd experimental groups and amounted to 7462 ± 71.50 and 7478 ± 63.57 kg, exceeding the same indicator in the control (7353 ± 86.93 kg) by 109 and 125 kg or by 1.71 and 1.96% (P<0.05). The mass fraction of fat in milk in the heifers of the 1st (3.96 ± 0.02%) and 2nd (4.07 ± 0.01%) experimental groups turned out to be 0.16 and 0.27% higher than in control (3.8 ± 0.04%).

The highest mass fraction of protein in milk for 305 days of lactation was observed in the 2nd experimental group - 3.12 ± 0.005%, that is, it turned out to be higher than the same indicator of the heifers of the 1st experimental (3.08 ± 0.003%) and control (3.05 ± 0.01%) groups of 0.04 and 0.07%.
Table 5. Effects of PS-7 and Prevention-N-C biopreparations on productive qualities of heifers.

| Indicator                       | Control   | 1 experimental | 2 experimental |
|---------------------------------|-----------|----------------|----------------|
| Milk yield for 305 days of lactation, kg | 7353±86.93 | 7462±71.50     | 7478±63.57     |
| Mass fraction of fat, %         | 3.8±0.04  | 3.96±0.02      | 4.07±0.01      |
| Mass fraction of protein, %     | 3.05±0.01 | 3.08±0.003     | 3.12±0.005     |
| Lactose, %                      | 4.12±0.04 | 4.27±0.03      | 4.31±0.05      |
| Dry matter, %                   | 12.2±0.02 | 12.33±0.02     | 12.43±0.01     |
| MSNF – milk solids non-fat, %   | 7.97±0.10 | 8.25±0.14      | 8.49±0.11      |
| Mineral substance, %            | 0.72±0.07 | 0.73±0.08      | 0.73±0.05      |
| Density, °A                     | 28.39±0.26 | 28.63±0.21     | 29.11±0.11     |
| Acidity, °T                     | 16.32±0.11 | 16.39±0.09     | 16.31±0.01     |

Notes: Differences between each experimental group and controls: * P<0.05; ** P<0.01.

The prevention of transport stress of imported heifers by the application of PS-7 and Prevention-N-C biopreparations appeared as a feasible approach, since an improvement in the physicochemical parameters of milk was observed. At the same time, the requirements of the Technical Regulations of the Customs Union “On Food Safety” of the TR CU 021/2011 and the Technical Regulations of the Customs Union “On the safety of milk and dairy products” TR CU 033/2013 were satisfied.

4. Conclusion
Intramuscular injection of imported heifers with PS-7 and Prevention-N-C biological products in a dose of 10 ml 7 days before transportation and 2 days after delivery alleviate transport stress by activating non-specific protective mechanisms of the body, contributing to improved reproductive and productive traits in heifers.

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