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## **USE OF LOW-INTENSITY LASER RADIATION IN REHABILITATION OF HYPOTROPHIC CALVES**

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### **ABSTRACT**

The effect of low-intensity laser radiation on the biochemical, immune and clinical status of hypotrophic calves was studied. The studies were carried out in 2 groups (test and control ones) of 12 animals with symmetric moderate hypotrophy. The calves in the test group underwent low-intensity laser irradiation of blood within the red spectral range (wave length of 630 nm). The animals of the control group (intact) were not treated. Clinical observations of the calves were carried out during 2 months, taking into account the incidence, duration and severity of the course of the disease, as well as the calves' weight gain. Blood sampling for biochemical and immunological studies was performed on the 1st and the 21st days of the calves' life. On the first day the biochemical status and natural resistance in the animals of the both groups did not differ. On the 21st day, the calves of the test group had higher levels of glucose, pyruvic acid, vitamin E, Blood Serum Complement Activity (SCA), Blood Serum Lysozyme Activity (SLA), Leucocyte Phagocytic Activity (LPA), Phagocytic Number (PN), Phagocytic Intensivity (PI), and lower levels of lactic acid, middle molecules and ectoglobular hemoglobin in comparison with the control group. The treatment of the animals with low-intensity laser radiation positively affected their clinical state and productivity. The calves in the test group had mild forms of gastrointestinal diseases with a shorter course duration, and the increase in body weight was significantly higher compared with the control group. The positive effects of low-intensity laser radiation on biochemical status, natural resistance and clinical condition of hypotrophic calves were established, which allows us to recommend it for their rehabilitation.

**Key words:** *calves, antenatal hypotrophy, low-intensity laser radiation, biochemical status of blood, natural resistance.*

### **INTRODUCTION**

In specialized milk production farms of the Russian Federation antenatal pathology (congenital liver disease, antenatal hypoxia, hypotrophy) is registered in 9-21% of the newborn calves. Antenatal hypotrophy occurs when the fetus has insufficient supplies of oxygen, nutrients, energy and biologically active substances. In order to maintain the vital activity of the fetus, necessary substances are redistributed to the

benefit of its vital organs. As a result, the development of the musculoskeletal system, the respiratory system and the gastrointestinal tract (and, in severe cases, the liver and the vascular system) is suppressed [Shakhov, 2013].

The perinatal development disorder of calves adversely affects the formation of the immune status and is manifested by immunodeficiency, which is accompanied by the instability of the natural resistance indicators dynamics and their adaptive immunity, which leads to the infection of animals with various pathogens and the occurrence of gastrointestinal and other diseases. The aggravation of hypotrophy in newborn calves results from the decrease in the adaptive capacity of their body, the accumulation of intermediate and final metabolites, the membrane structures disorder, the endogenous intoxication and metabolic acidosis. The subsequent growth and development disorders of such animals and their high disease rate call for rehabilitation activities [Alyokhin, 2013]. To activate the protective mechanisms of the body, various means and methods are used that are divided into three groups based on their origin: biological, originating from cells and tissues of living organisms (animals, humans, microbes, plants), chemical (natural and synthetic) and physical (radiation energy, ultrasound, magnetic field and others). In this aspect, non-medicamentous immunocorrection should be considered. It can be used to treat severe diseases, drug therapy resistance or side effects. Non-pharmacological methods are environmentally safe, since no foreign potentially dangerous chemical substances are introduced into the animal's body. Non-medicamentous immunomodulation includes low-intensity laser radiation [Kataranov, 2005, Golubtsov, 2009].

Research objective was to study the effect of low-intensity laser radiation on the biochemical status and natural resistance of hypotrophic calves and the possibility of using it for their rehabilitation.

### MATERIALS AND METHODS

The research was carried out at Agrofirma "Grachevskoe" farm in the Usman district of the Lipetsk region on calves obtained from the red-and-white «Voronezhky» cows. Animals selected for the experiment had the syndrome of the symmetric moderate hypotrophy: low body weight ( $27,3 \pm 0,3$  kg), hypothermia tendency (body temperature after 12 hours after birth  $38,4 \pm 0,05^{\circ}\text{C}$ ), moderate tachycardia ( $144,0 \pm 3,75$ ), tachypnoe ( $39,5 \pm 0,50$ ), inhibition of physiological reflexes, etc.

The animals were divided into two groups by the analog method ( $n=12$ ). The calves of the control group were intact. The animals of the test group were exposed to the low-intensity laser radiation (LILR). For the procedure, "Matrix" laser therapeutic apparatus with a KO4 radiator (wavelength of 630 nm) attached was used. The calves had their hair cut in the jugular vein and were contact-scanned for 5 minutes with a slight soft tissue compression in the morning; the treatment course had 10 sessions with a 48 hours interval. The radiation power in the first session was 2,5 mW. During the next six sessions, it was increased by 2,5 mW each time, and then kept at the level of 15 mW. When choosing the mode and dose of

radiation, general recommendations on the use of physiotherapeutic methods were followed, as well as the method for evaluating the effect of low-intensity laser radiation of blood on the animal body [Golubtsov, 2014]. Clinical observations of the calves were carried out for 2 months, taking into account the incidence, duration and severity of the course of the disease, and the calves' weight gain. Blood sampling for biochemical and immunological studies was performed on the 1st and the 21st days of the calves' life. To determine the etiology of gastrointestinal diseases, feces were studied for bacteriological culture.

Conventional methods were applied to study the blood morphological composition, the neutrophils absorption activity, the lysozyme, complementary and bactericidal activity of the blood, the vitamin E concentration, glucose, pyruvic and lactic acid, the middle molecules content (MMC), ectoglobular hemoglobin (EGH), the erythrocyte membrane modification coefficient (EMM), bacteriological studies of feces. Statistica v6.1 applications were used for statistical processing of the obtained data, and the Student's t-test – for reliability assessment.

### RESULTS AND DISCUSSION

The clinical status of the hypotrophic calves in the control and test groups in the first days of life was almost identical. They independently rose after  $2,6 \pm 0,18$  and  $2,8 \pm 0,12$  hours, the manifestation of the sucking reflex was recorded after  $2,0 \pm 0,27$  and  $2,3 \pm 0,37$  hours, the body temperature was at  $38,9 \pm 0,09$  and  $38,8 \pm 0,14^\circ\text{C}$ , the pulse was  $120,0 \pm 2,0$  and  $120,7 \pm 2,0$  / min, the respiratory rate was  $38,0 \pm 2,0$  and  $36,8 \pm 0,5$  per minute, and the meconium was released after  $9 \pm 1,5$  and  $10,0 \pm 1,5$  hours, respectively.

The blood chemistry values in the calves of the both groups in the first day of life did not differ (Table 1).

Table 1. The blood chemistry values in hypotrophic calves on the first day of life

Value	Unit of measurement	Control group	Test group
Glucose	M/L	$6,99 \pm 0,30$	$7,01 \pm 0,43$
Pyruvate	mM/L	$180,0 \pm 20,8$	$178,0 \pm 19,0$
Lactate	M/L	$3,3 \pm 0,17$	$3,4 \pm 0,21$
EGH, g/L	g/L	$1,62 \pm 0,04$	$1,68 \pm 0,02$
Vitamine E	$\mu\text{M/L}$	$4,97 \pm 0,69$	$4,36 \pm 0,63$
MMC <sub>237nm</sub>	cond. unit	$0,61 \pm 0,011$	$0,76 \pm 0,005$
MMC <sub>254nm</sub>	cond. unit	$0,36 \pm 0,001$	$0,36 \pm 0,002$
EMM		$1,60 \pm 0,02$	$1,62 \pm 0,01$

**Note:** \* $p \leq 0,05$ ; \*\* $p \leq 0,01$ ; \*\*\* $p \leq 0,001$ ; numerator – control group, denominator – test group.

On the day 21st the glucose content in the animals of the test group, compared with the control group, was 25,0% higher, the pyruvic acid content - 30,2 higher, and the lactic acid content - 9,9% lower. The glycolysis activation with the

dominance of the aerobic pathway is confirmed by the optimal lactate/pyruvate ratio ( $12,3\pm 3,33$ ). In the intact calves, it was 30,5% higher ( $17,7\pm 0,53$ ), indicating a higher activity of anaerobic glycolysis (Table 2).

Table 2. The blood chemistry values in hypotrophic calves on the twenty-first day of life

Value	Unit of measurement	Control group	Test group
Glucose	M/L	$4,36\pm 0,27$	$5,45\pm 0,43^*$
Pyruvate	mM/L	$97,0\pm 4,98$	$126,3\pm 8,13^{**}$
Lactate	M/L	$1,72\pm 0,06$	$1,55\pm 0,04^*$
EGH, g/L	g/L	$1,04\pm 0,01$	$0,35\pm 0,02^{***}$
Vitamine E	$\mu\text{M} / \text{L}$	$6,43\pm 1,08$	$9,20\pm 0,62^*$
MMC <sub>237nm</sub>	cond. unit	$1,17\pm 0,024$	$0,50\pm 0,013^{***}$
MMC <sub>254nm</sub>	cond. unit	$0,34\pm 0,004$	$0,21\pm 0,021^*$
EMM		$1,54\pm 0,03$	$1,32\pm 0,02^{***}$

Note: \* $p\leq 0,05$ ; \*\* $p\leq 0,01$ ; \*\*\* $p\leq 0,001$ ; numerator–control group, denominator–test group.

In the pathogenesis of the auto-intoxication, an important role belongs to the defect of the membrane structures. The indirect reflection of the membrane structures state are the values of ectoglobular hemoglobin (EGH), the erythrocyte membranes modification coefficient (EMM), as well as the content of vitamin E and middle molecules content (MMC).

The level of the ectoglobular hemoglobin in the hypotrophic calves was 2,7-2,8 times higher than the values typical of healthy normotrophic calves (0,60 g/l) on the first day of life. On the day 21st, the intact hypotrophic calves showed a decrease in the destructive phenomena in the erythrocyte membranes, as indicated by a 36,0% decrease in EGH, while it did not exceed the norm (0,40 g/l) in the animals of the test group, due to the restoration of the membrane structure.

Of the calves with mild and moderate congenital hypotrophy, a distinctive imbalance of the autonomic nervous system with the sympathetic division dominance is typical, which is confirmed by a higher level of adrenoceptor activity on the surface of erythrocyte membranes. Our studies showed that the erythrocyte membranes modification coefficient under the action of epinephrine in the experimental calves at the diurnal age was 1,6 (1,1-1,4). The EMM in the animals of the control group decreased with the increase in age, but remained higher (1,54) than in healthy calves (1,4). The low-intensity laser radiation of calves' blood promoted the normalization of the membrane-receptor complexes of erythrocytes, and as a result the EMM decreased to the physiological values (1,32). The low-intensity laser radiation decreased the number of the middle molecules by 38,2% ( $\lambda=254$  nm) and 57,3% ( $\lambda=237$  nm) in the animals, which indicates the decrease in the level of both metabolic and resorptive endogenous intoxication. At the same time, the resorptive auto-toxication progressed in the intact calves with age (1,17 cond. units).

One of the indicators reflecting the state of the blood antioxidant system is the concentration of vitamin E. Its content in the calves of the control group increased by 29,4%, and in the animals of the test group - by 2,1 times on the day 21st. The higher level of vitamin E in the animals treated with the low-intensity laser radiation lends an indirect evidence of the decrease in the intensity of the processes of lipid peroxidation and the attenuation of the destructive processes in their body membrane structures.

Studying of the natural resistance in the calves of the both groups on the first day revealed no significant differences in values (Table 3).

Table 3. The natural resistance values in hypotrophic calves on the first day

Value	Unit of measurement	Control group	Test group
SBA	%	77,3±2,19	74,2±2,79
SCA	% hem.	15,4±0,30	16,1±0,51
SLA	mg / ml	1,6±0,05	1,8±0,11
LPA	%	78,6±3,81	76,0±4,34
PN		6,20±0,75	6,46±0,77
PI		7,8±0,64	7,37±0,65

Note: \*p≤0,05; \*\*p≤0,01; \*\*\*p≤0,001; numerator – control group, denominator – test group.

Most of the natural resistance values in the animals of the control group decreased in comparison with the background data on the day 21st : SBA by 5,5%, SCA by 8,9, LPA by 3,4, PN and PI by 6,9 and 1,8% while SLA increased by 31,3%. The calves under the influence of the radiation had a more significant increase in SLA (2 times), as well as LPA by 3,4%, PN and PI by 22,3% and 23,5%, respectively, and a less prominent decrease in SBA (by 1,4%) and SCA (2,3%). Comparing the natural resistance values in the intact animals and the test group calves within the indicated period, it should be noted that the latter had 2,1 times higher SCA, 1,7 times higher SLA, 4,2% increase in LPA, 36,9% and 18,8% increase in PN and PI respectively, which indicates a stimulating effect of the radiation on the nonspecific protection (Table 4).

Table 4. The natural resistance values in hypotrophic calves on the twenty- first day

Value	Unit of measurement	Control group	Test group
SBA	%	71,8±2,89	72,8±1,74
SCA	% hem	6,5±0,60	13,8±0,62 <sup>***</sup>
SLA	mg / ml	2,1±0,46	3,6±0,13 <sup>**</sup>
LPA	%	75,2±1,78	79,4±1,04 <sup>*</sup>
PN		5,77±0,67	7,90±1,02 <sup>*</sup>
PI		7,66±0,42	9,1±0,57 <sup>*</sup>

Note: \*p≤0,05; \*\*p≤0,01; \*\*\*p≤0,001; numerator–control group, denominator–test group.

The clinical studies in the calves of the both groups recorded gastrointestinal pathology during the colostrum period, but the severity and the duration of the

disease progression varied. In all the animals of the control group during the colostrum period, the disease progressed in a severe form for 4-6 days, with the first signs registered on the second day in 50% of the cases. From the feces of the diseased calves, the enteropathogenic *E. coli* of the serovariants 02; 04; 026; 0103; 0138; 0141 were excreted. The relapse of the gastrointestinal diseases was registered on the day 9th of life; the duration of the pathology was an average of 6 days. From the feces of the sick animals, *E. coli* of the serovariants: 02; 026; 033; 0103; 0138; 0115 and the coronavirus gene were excreted. The mean daily weight gain during 2 months was  $340,7 \pm 20,0$  g. Gastrointestinal diseases were also recorded in all the animals of the test group, but they proceeded in a mild form for 3 days. Diarrheal syndrome was recorded in 50% of the animals from the second day of life, with an average duration of 2 days. The diarrheal syndrome manifested itself on the days 4th-6th of life with an average duration of 3 days in 50% of the animals. From the feces of the sick animals, enteric-pathogenic *E. coli* serovariants 02; 04; 026; 0103; 0138; 0141 were excreted. The mean daily weight gain during 2 months was  $550,5 \pm 24,0$  g.

### CONCLUSION

Low-intensity laser radiation of blood in hypotrophic calves increases the natural nonspecific resistance, the stability of the membranes of the body cellular elements, it stimulates synthetic and metabolic processes, reduces auto-intoxication, which allows us to recommend it for rehabilitation activities.

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