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EFFICIENCY OF INTRAVASCULAR LASER BLOOD IRRADIATION IN CATTLE WITH INFLAMMATORY SURGICAL PATHOLOGY

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The laboratory studies results of intravascular laser blood irradiation (ILBI) influence on the bodies of bulls, subjected to jugular veins resection and castration, and cows with papillomatous dermatitis in the area of fingers are presented. After ILBI application similar changes in blood serum of experimental animals were established for different pathological processes. The tendency of ASAT, AP activity and C-reactive protein amount reducing to the norm was revealed, indices growth of non-specific immunological reactivity: serum BSBA and BSLA and opsono-phagocytic activity of blood neutrophils was determined. In the experimental group bulls, one or two days earlier the wound healing and the absence of tissue edema around the affected areas were observed.

Key words: intravascular laser blood irradiation, inflammatory processes, cows, bulls.

The study is a fragment of the research project "Pathogenesis study and development of pathogenetic methods for the inflammatory processes treatment in surgical diseases of animals" (state registration number 11117U004721, 2017-2027) and is part of the scientific subject "Application of low-intensity laser irradiation for treatment and correction of inflammatory processes in productive animals" (state registration number 0113U005191).

Since the 1960s, physiotherapeutic methods of immune correction and laser therapy, in particular [5], have started to be implemented into the clinical practice of veterinary medicine. It contributes to the increased body resistance, accelerates recovery of animals and eliminates or reduces the need for antibiotics. To date, there are only a few publications on the study of the ILBI effect on the surgical pathology course in animals of different species. After application of this method in pigs, a reduction in the damaged tissue swelling and an enhancement in the process of purulent necrotic masses sloughing was observed due to an increase in the amount of tissue plasminogen activator and fibrinolytic activity. During the literary search, we found individual reports on the positive ILBI effect in the treatment of cattle with inflammatory pathology, as well as horses [9, 10] and dogs [6]. This suggests that the above method for treating animals with inflammatory pathology has been poorly studied. Therefore, development of methods for treating animals with inflammatory pathology, taking into account the in-depth pathogenesis study, is of the significant scientific and practical importance.

The purpose of paper was to substantiate clinically and experimentally the feasibility of using ILBI for treating animals with different inflammatory surgical pathologies.

Materials and methods. Clinical studies were carried out on the cattle stock of red and black-and-white Ukrainian dairy breeds in the conditions of three dairy farms in the Poltava region: TOV "Dukla" of the Commercial Dairy Farm (CDF) in the village of Ivashky, the Poltava region; "Astarta-Kyiv" Agro-industrial holding, VP "Gogolevo", CDF No.1, the village of Gogolevo, Shishaky District, and the "Trostanets" KS of the Poltava District during 2011-2016. Laboratory studies were carried out on the basis of accredited laboratories of the veterinary and biological profile of Poltava and the educational and scientific laboratory at the Surgery and Obstetrics Department of PSAA. The studies included two stages.

Experiment 1: The ILBI effect on healing experimental wounds caused by the jugular vein resection (first stage) and castration (second stage) was studied under laboratory conditions in twelve bulls weighing 180 ± 20 kg. They were divided into two groups by the analogy principle: the control and the experimental groups with six animals in each one. Animals were subjected to the local treatment: a single application of Chemi Spray (INVESA, Spain, Barcelona) to the wounds.

Six animals of the experimental group in the postoperative period were additionally subjected to ILBI five times with a 22-24 hour interval. The duration of one session was 15 minutes. For this purpose, the "Matrix-VLOK" apparatus with KL-VLOK radiation head with a wavelength of $0.63 \mu\text{m}$ was applied. The power at the end of the fiber waveguide was 1.5-2.0 mW.

The animals were subjected to the constant clinical observation and a regular examination of tissues at the wound sites, paying attention to pain in palpation, hiatus, bleeding, swelling and the presence of exudation.

Phagocytic activity of neutrophils (FA) and phagocytic index (FI) in the animals' blood were first determined after operations before irradiation, the second and the third times - at intervals of 14 days by the described method [1]. As a test culture, a freshly cultured *Staphylococcus aureus*, strain 219-P, was used. Blood samples were taken from the jugular vein and stabilized with heparin [4].

Blood serum bactericidal activity (BSBA) was determined according to the described method [2], blood serum lysozyme activity (BSLA) - in the modification of IECVM [8]. As test cultures, *Escherichia coli* (strain O-139) and *Micrococcus lysodeikticus* (strain 2655) were used respectively. Content of C-reactive protein was determined by means of the latex test using the sets produced by TOV NVL "Granum" company, Kharkiv.

The BA 88 biochemical analyzer, Mindray (China), was applied for biochemical blood serum test. The ASAT (alanine aminotransferase), ALAT (aspartate aminotransferase) activity (Reitman-Frankel's method), AP (alkaline phosphatase, by diethanol amine (DEA)-buffer method), total calcium content (by photometric method) and total protein (by biuret reaction) were determined applying the sets produced by TOV NVP "Filicet-Diagnostika", Dnipro.

Experiment 2: involved 850 cattle animals of CDF (dairy farm) No.1, the village of Gogolevo, Shyshaky District and 199 cattle animals of CDF, Ivashky village, the Poltava region. Clinical studies were performed to detect purulent lesions in the distal part of the limbs according to the generally accepted method [3]. Taking into account the signs, the pathologies were classified as superficial purulent pododermatitis, laminitis, purulent wounds, purulent arthritis of the finger joints, punctures of the soles, finger papillomatous dermatitis, erosion of the heel, ulcer of the sole. The forms of movements were estimated daily, and signs of purulent-necrotic processes in the area of fingers in cows were revealed, simultaneously the indices of trias, scar reduction, etc. were controlled.

The movements evaluation in cows was carried out on a solid, even surface of the ground without obstacles. Particular attention was paid to the position of the back while standing and motion according to the method described [7]. In the walking evaluation, it was considered the norm (1 point) if the cow was standing and going with a flat back and was making sure long steps. Weak lameness (2 points) was established when the animal was standing with its back flat, while at walking it was hogged. Walking was slightly disturbed. Moderate lameness (3 points) - the cow was standing and walking with pronounced hogged back and making short steps. The 4 points lameness meant that the cow's back was always clearly hogged, the steps were cautious, especially basing upon the affected limb. In acute lameness (5 points), the cows had a clearly pronounced hogged back, the animal was moving reluctantly and tried not to step on the affected limb.

To detect superficial purulent pododermatitis in cows, every three days after changing the dressings, attention was paid to reddening, inflammatory edema, local temperature, the presence of pain at percussion and palpation of the affected area, and the nature of the purulent exudate, discharging while hooves debridement.

In each farm, one control group and one experimental group ($n = 5$) were formed on the analogues principle of clinically healthy animals and those with purulent inflammation in the fingers area. They were treated by the method described above [10]. To do this, each animal was fixed in a standing position and the diseased limb was fixed to the side housing or the ox-stall. The distal parts of the limbs were washed with warm water and the dirt and purulence were removed from the hooves with a hoof knife. A thorough surgical treatment of purulent lesions was performed and a gauze dressing medicated with a 5% solution of copper sulfate was applied. Dressing and of the drug application were repeated five times with a three days interval.

An animal had its head fixed in order to have free access to the jugular vein. Its hair was shaved and its skin was disinfected. The jugular vein was injected with a single-use needle, which was connected with a single-use waveguide with the KL-VLOK head, emitting infrared light with the wavelength of 0.63 μm and the power of 2.0 mW. This procedure was repeated five times for 15 minutes with one-hour interval. The "Matrix-VLOK" apparatus (fig. 1) was used for irradiation. In the control group, the local treatment only was used.

Blood and serum samples were taken and examined prior to treatment on the 1st, 5th, and 15th days of treatment using the same techniques as in Experiment 1.



Fig.1. Treating a cow with ILBI.

Digital material was processed by the variation statistics methods with a personal computer using the MS Excel software and the tables by R.B. Strelkov (1966). The probability of differences between the indices was assessed according to Student's criterion. The difference between the two values was considered probable for $p < 0.05$; $p < 0.01$; $p < 0.001$.

Results of the study and their discussion. In bulls of the both groups, at the end of the first day after the suturing around the wounds formed during the jugular vein resection, a pronounced tissue swelling at the

distance of 0.6 ± 0.05 cm from their edges, their pasty consistency, skin reddening were observed due to active hyperemia and pain.

Indices of the tissues state around the bulls' wounds are presented in table 1. It is evident that signs of inflammation in the jugular vein area in animals of the control group were found within the period up to 4 ± 0.1 and those in the experimental group - up to 3 ± 0.1 ($p < 0.01$) days.

Table 1

Indices of the tissues state in experimental wounds areas in bulls after the ILBI application
($M \pm m$) ($n = 6$)

Indices	Control group	Experimental group
Jugular veins resection		
Recovered, %	100.0%	100.0%
Subsidence of tissue edema around the wounds edges, days	4.0 ± 0.1	$3.0 \pm 0.1^{**}$
Wound edges adhesion, days	8.0 ± 0.1	$7.0 \pm 0.1^{**}$
Castration		
Recovered, %	66.6%	100%
Subsidence of tissue edema around the wounds edges, days	9.0 ± 0.2	$7.0 \pm 0.1^{***}$

Note: $^{**} p < 0.01$; $^{***} p < 0.001$ compared to the control group animals.

With edema reducing, there was a decrease in other signs manifestations of inflammatory tissue reaction: pasty consistency, pain, increased local temperature and hyperemia.

Two days after, in animals of the both groups, a small amount of clear fluid discharge from the wound was observed, the fluid being slightly yellowish in color. As a result of its drying, yellowish or brown skin pells were formed on the skin surface.

On the fourth day, in one control group, a small amount of inflammatory exudate was detected through the sites of tissue punctures during the dressing application. It dried up on the skin around the thread and formed a pell of yellow color. In the experimental group, at the place of the thread entry into the soft tissue, exudation was not recorded.

In the control group animals, up to 8 ± 0.1 days, a narrow scar of the granulation tissue was formed on the wound's surface. In the experimental group animals, the above reaction was detected one day earlier, and the sutures threads were removed much easier.

After castration, in the bulls, fluctuations of the trias indices were observed within the normal range for 14 days. On the first day, the clinical condition of the both groups animals was similar: slight depression, acampsia, appetite preserved. The skin of the scrotum was bright pink, shiny and tense (hydrophobic), and the wall was of pasty consistency, thickened. The wounds' edges were drawn to its middle. The size of the perifocal inflammatory edema around the incision was 7.0 ± 0.03 cm. The wounds, pain and the local temperature increase were clearly pronounced.

On the second day, in the both groups animals, the larger sizes of the scrotum were observed than before the castration. The skin was bright pink, shiny and tense. The edges of the wound are glued with fibrin. In the experimental group animals treated with ILBI, less severe pain was observed compared to that of the control group. The wound was gaping without bleeding. One control animal in the postoperative period was recorded to have a significant diffuse edema around the surgical wound, and on the third day, a viscous exudate discharge was observed with impurities of whitepurulence.

On the third day, in experimental group animals, pain at palpation was less pronounced than in the control group. In the both groups animals, swelling of tissues and wound gaping without bleeding and exudation were expressed.

Starting from the fourth day, in the experimental group animals, pain at palpation disappeared, wound gaping became less pronounced due to the reduced tissue edema. Bleeding and leakage from the wounds were absent. On the fifth day, no pain reaction was detected. The control group animals were still having tissue edema.

On the sixth and seventh day, in the both groups animals, appetite was normal. The volume of scrotum was reduced, the skin has a pink color, bright pink around the wounds with a certain tension. The scrotum wounds were reduced in size and covered with crusts. In the experimental group animals, on the 7 ± 0.1 day ($p < 0.001$), the scrotum reduced in volume, the skin was not tense at palpation, pink and bright pink around the wound, slightly tense. The scrotum wounds reduced in size and were covered with crusts. In the control group, similar indices were recorded on the 9.0 ± 0.2 day.

Results of the bulls' blood biochemical studies are presented in tables 2 and 3.

Table 2

Results of biochemical studies on blood serum samples of bulls, subjected to the veins resection, before and after the ILBI application, $M \pm m$, $n = 6$

Indices	Animals					
	before ILBI application		after ILBI application			
			the 1st day		the 14th day	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
ASAT, u/l	42.4 $\pm$ 1.3	41.7 $\pm$ 1.4	72.7 $\pm$ 3.9	83.3 $\pm$ 2.0*	830 $\pm$ 11.6	68.7 $\pm$ 3.4*
ALAT, u/l	26.2 $\pm$ 1.1	25.7 $\pm$ 1.0	36.7 $\pm$ 3.4	39.7 $\pm$ 6.7	35.0 $\pm$ 2.9	35.7 $\pm$ 4.2
AP, u/l	108.6 $\pm$ 2.4	110.6 $\pm$ 1.3	285.5 $\pm$ 3.8	255.0 $\pm$ 12.3*	227.3 $\pm$ 10.8	148.3 $\pm$ 13.6**
Total protein, g/l	76.2 $\pm$ 1.1	77.5 $\pm$ 1.9	76.0 $\pm$ 0.8	70.3 $\pm$ 0.4***	67.7 $\pm$ 0.4	66.0 $\pm$ 1.9
Cholesterol, mmol/l	2.4 $\pm$ 0.2	2.6 $\pm$ 0.4	1.63 $\pm$ 0.01	1.3 $\pm$ 0.1**	1.1 $\pm$ 0.02	0.97 $\pm$ 0.1

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to the control group animals.

As it can be seen in table 2, the studied indices had certain fluctuations in the experimental and control groups animals, but not in the reliable limits. In blood serum of bulls having an experimental tissue injury in the neck area, the increased activity of ASAT, AP, and reduced cholesterol were detected. In bulls subjected to ILBI, on the next day, there was reliably ($p < 0.05$) the highest index of ASAT activity (83.3 ± 2.0 u/l), compared to that of the control group (72.9 ± 3.9 u/l) and on the 14th day it was reliably lower - 68.7 ± 3.4 u/l ($p < 0.05$).

Table 3

Results of biochemical studies on the blood serum of castrated bulls after the ILBI application, $M \pm m$, $n = 6$

Indices	Animals			
	the 1st day		the 14th day	
	Control group	Experimental group	Control group	Experimental group
ASAT, u/l	83 $\pm$ 4.6	68.7 $\pm$ 3.4*	75.3 $\pm$ 2.1	62.3 $\pm$ 3.4*
ALAT, u/l	35.0 $\pm$ 2.9	35.7 $\pm$ 4.2	33.3 $\pm$ 2.9	45.3 $\pm$ 4.3*
AP, u/l	227.3 $\pm$ 16.8	148.3 $\pm$ 13.6**	219.7 $\pm$ 7.5	195 $\pm$ 4.5*
Total protein, g/l	77.7 $\pm$ 0.4	76.0 $\pm$ 2.9	74.3 $\pm$ 5.9	75.3 $\pm$ 3.4
Cholesterol, mmol/l	1.2 $\pm$ 0.02	0.95 $\pm$ 0.2	1.47 $\pm$ 0.3	1.2 $\pm$ 0.1*

Note: * $p < 0.05$; ** $p < 0.01$ compared to the control group animals.

There was a tendency to a reliable ($p < 0.05 - 0.01$) decrease in the AP activity in the treated bulls after the first day up to 255.0 ± 12.3 u/l and after the 14th day up to 148.3 ± 13.6 u/l. In the control group, the data were 285.5 ± 3.8 u/l and 227.3 ± 10.8 u/l respectively. In addition, after the first day of treatment, in the animal blood serum, the amount of total protein decreased up to 70.3 ± 0.4 g/l and the cholesterol content – up to 1.3 ± 0.1 mmol/l. In the control group of animals, these indices were 76.0 ± 0.8 g/l and 1.63 ± 0.01 mmol/l respectively.

Based on the data of table 3, we can concluded that in the blood serum of experimental bulls on the first and the 14th days, the ASAT and AP activity was reliably ($p < 0.05-0.01$) less, but only on the 14th day ALAT activity was greater and the cholesterol level was lower, compared to those in the control animals.

The results of the neutrophils phagocytic activity study in the blood of bulls are presented in table 4.

The data in table 4 indicate that after the first and the second operations, in the blood of the experimental group bulls, on the 14th day, PhA and PhI levels were reliably ($p < 0.05-0.001$) elevated compared to the controls.

Table 4

Indices of the bulls' blood neutrophils phagocytic activity before and after the operations and the ILBI application, $M \pm m$, $n = 6$

Indices	Before veins resection		After veins resection (the 1st operation)				Castration (the 2nd operation)	
			the 1st day		the 14th day		the 14th day	
	Contr. group	Exper. group	Contr. group	Exper. group	Contr. group	Exper. group	Contr. group	Exper. group
PhA, %	43.8 $\pm$ 0.6	42.9 $\pm$ 0.7	41.5 $\pm$ 1.2	40.4 $\pm$ 0.6	38.5 $\pm$ 0.5	44.5 $\pm$ 1.2***	39.1 $\pm$ 0.6	44.8 $\pm$ 1.2**
PhI, abs. number	4.8 $\pm$ 0.1	4.9 $\pm$ 0.3	3.1 $\pm$ 0.2	3.5 $\pm$ 0.1	3.0 $\pm$ 0.1	3.8 $\pm$ 0.2**	2.9 $\pm$ 0.2	3.7 $\pm$ 0.2***

Note: ** $p < 0.01$; *** $p < 0.001$ compared to the control group animals.

Cows with papillomatous dermatitis used to protect the damaged limb from the load, often raised it, performed shaky movements. On the move, seven animals in the both groups showed the third, and three animals – the fourth degree lameness.

In most animals, in the perioplic ring skin, clearly limited rounded or oval lesions were observed, resembling skin areas with the epidermis lost. They were pink in color without any discharge, very painful to the touch. The surface of the defect was filled with granulation tissue, protruding above the skin surface, forming funguslike formations. Rounded, bulging, painful granulation mass, easily bleeding. These injuries were limited by a light-gray epithelial rim. The adjacent areas of the skin, as a rule, did not undergo pathological changes, the regrowth of long hair was observed in them.

After the ILBI application while treating limb lesions in all the five cows, signs of lameness disappeared after 9.6 ± 0.8 days ($p < 0.05$), the wound surface was cleaned on the 7.8 ± 0.8 day, signs of their granulation and epithelization were noticed on the 10.2 ± 0.8 day and the complete clinical recovery – on the 12.6 ± 0.8 day. These figures in four (80.0%) of the five animals in the control group were 12.7 ± 0.8 days, 9.7 ± 0.8 , 11.2 ± 0.8 and 14.5 ± 0.8 days respectively.

The data obtained show that in the blood serum of the experimental group cows, on the fifth and fifteenth days, a reliably lower activity of ASAT was observed compared to that of the control group. The increase of AP activity and cholesterol contents was only recorded on the 15th day.

In the blood serum of the cows, which were subjected to ILBI, the BSBA, BSLA, PhA and PhI contents were reliably ($p < 0.05-0.001$) increased on the 5th and the 15th days. The index of PhI was elevated one day after the ILBI application.

Attention should be drawn to the dynamics of CRP (C-reactive protein) content. Thus, until the end of treatment on the 15th day, in animals of the experimental group it had disappeared, whereas in animals of the control group (though its reduction was recorded by 3.4 times), it remained at the level of 3.6 ± 1.3 mg/l.

Conclusion

Thus, in all six bulls, subjected to ILBI after jugular vein resection, the tissues edema disappeared and the wounds edges adhered on the 3rd and on the 7th day, respectively. The same pattern was observed in only four of the six animals in the control group and one day later. In all the experimental group bulls after the castration, tissue swelling disappeared after seven days, and in four out of the six control group animals - nine days later.

In blood serum of bulls, after resection of jugular veins and castration, on the first and the 14th day after the ILBI application, there was a reliable decrease in the ASAT and AP activity compared to the control group animals, but it was higher compared to the indices before the operation. On the 14th day from the beginning of treatment, in blood of the bulls, subjected to the jugular veins resection and castration, the PhA and PhI indices were found to be reliably higher.

In the blood serum of cows with papillomatous dermatitis, a reliable decrease compared with the control animals in the ASAT activity was also observed after 5 and 15 days after the ILBI application, and only after 15 days - in the activity of AP. In these animals, after 5 and 15 days, the reliable increase in BSBA and BSLA was found in the blood serum, and the increase of PhA and PhI in the blood.

Consequently, after the ILBI application, in bulls and cows with different pathological processes, there was a similar reaction: the tendency to the decreased activity of ASAT, AP, and the C-reactive protein content to the norm, and to the increase of non-specific immune reactivity indices: BSBA and BSLA in the blood serum and opsono- phagocytic activity of blood neutrophils.

This indicates positive effects of the ILBI on the body of cattle and the necessity of further studying the ILBI effect on the physiological and immunological parameters and animal productivity in the norm and in pathology.

Prospects of further research lie in the fact that the above data prove the necessity of further studying the effect of ILBI on physiological parameters and animal productivity in normal and pathological processes of different nature.

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Реферати

ЭФЕКТИВНОСТЬ ВНУТРИСУДИННОГО ЛАЗЕРНОГО ОПРОМЯНЕНИЯ КРОВИ У ВЕЛИКОЙ РОГАТОЙ ХУДОБИ ИЗ ЗАПАЛЬНОЙ ХИРУРГИЧНО-ПАТОЛОГИИ

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З 2007 по 2016 рр проводились клінічні дослідження на поголів'ї великої рогатої худоби червоної і чорно-рябої українських молочних порід в умовах трьох молочно-товарних ферм Полтавської області: ТОВ «Дукла» МТФ «Івашки» Полтавського району, Агропромхолдінг «Астарта-Київ», ВП «Гоголеве», МТФ №1 (с. Гоголеве) Шишацького району, і КС «Тростянець». Лабораторні дослідження були виконані на базі акредитованих лабораторій ветеринарно-біологічного профілю м.Полтави та навчально-наукової лабораторії кафедри хірургії та акушерства Полтавської державної аграрної академії. Крім того, на базі кафедри хірургії та акушерства ПДАА проведено два досліді, спрямованих на встановлення впливу ВЛОК на загоєння експериментальних ран. Клінічно було обстежено 1049 корів і 6 голів прооперованого молодняка великої рогатої худоби. За результатами досліджень обґрунтовано доцільність застосування внутрішньосудинного лазерного опром'янення крові за різних форм хірургічної патології у великої рогатої худоби, встановлено ефективність опром'янення крові по динаміці клінічних показників, а також біохімічних і імунологічних показників крові (сироватки).

Викладене вище переконливо доводить необхідність проведення подальших поглиблених досліджень, спрямованих на визначення доцільності використання інтраваскулярного лазерного опром'янення крові при лікуванні тварин із запальною патологією.

Ключові слова: внутрішньосудинне лазерне опром'янення крові, запальні процеси, бички, корови.

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ЭФФЕКТИВНОСТЬ ВНУТРИСОСУДИСТОГО ЛАЗЕРНОГО ОБЛУЧЕНИЯ КРОВИ У КРУПНОГО РОГАТОГО СКОТА С ВОСПАЛИТЕЛЬНОЙ ХИРУРГИЧЕСКОЙ ПАТОЛОГИЕЙ

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С 2007 по 2016 гг проводились клинические исследования на поголовье крупного рогатого скота красной и черно-пестрой украинской молочной пород в условиях трех молочно-товарных ферм Полтавской области ООО «Дукла» МТФ «Ивашки» Полтавского района, Агропромхолдинг «Астарта-Київ», ОП «Гоголево», МТФ №1 (с. Гоголево) Шишацкого района, и КС «Тростянец». Лабораторные исследования были выполнены на базе аккредитованных лабораторий ветеринарно-биологического профиля г. Полтавы и учебно-научной лаборатории кафедры хирургии и акушерства Полтавской государственной аграрной академии. Кроме того, на базе кафедры хирургии и акушерства ПГАА проводили два опыта, направленных на установление влияния ВЛОК на заживление экспериментальных ран. Клинически было обследовано 1049 коров и 6 голов прооперированного молодняка крупного рогатого скота. По результатам исследований обоснована целесообразность применения внутрисосудистого лазерного облучения крови при различных формах хирургической патологии у крупного рогатого скота, установленная эффективность облучения крови по динамике клинических показателей, а также биохимических и иммунологических показателей крови (сыворотки). Изложенное выше убедительно доказывает необходимость проведения дальнейших углубленных исследований, направленных на определение целесообразности использования интраваскулярного лазерного облучения крови при лечении животных с воспалительной патологией.

Ключевые слова: внутрисосудистое лазерное облучение крови, воспалительные процессы, бычки, коровы.

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