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BLOOD BIOCHEMICAL PARAMETERS IN TRANSFER FACTOR DONOR COWS DEPENDING ON SENSITIZATION SCHEME

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Abstract. *Research and development of means for effective prevention and treatment of diseases in animals are one of the priorities for modern veterinary science. Means based on the transfer factor are quite promising to solve these problems. One of the stages of obtaining a qualitative transfer factor specific to a particular disease is the sensitization of the body of donor animals. The purpose of this work was to investigate the blood biochemical parameters of donor cows after sensitization according to different schemes. The experiments were performed on cows of the Ukrainian black-spotted dairy breed, aged 4–5 years. Sensitization of pregnant cows was performed 1–1.5 months before calving with a concentrated formol-alum vaccine against salmonellosis of calves manufactured by the Kherson Biofactory. The vaccine was administered to the animals of the first experimental group one month before calving, one-time in a dose of 10 ml. Animals of the second experimental group 1.5 months before calving were two-time vaccine administered with an interval between injections of 10 days in doses of 10 and 15 ml. Studies have shown that in donor cows, which were two-time vaccine administered, there was an increase in hemoglobin content by 13% ($P<0.05$). There was also a decrease in glucose and creatinine content by 13–28% ($P<0.05$–0.01) in the blood serum of pregnant cows, which did not depend on the sensitization scheme, and a tendency to a decrease in total protein content. Regardless of the sensitization scheme of cows, an increase in serum aminotransferase activity was observed by 1.3–1.5 times ($P<0.05$–0.001), and if alanine aminotransferase activity increased mainly with a single injection of the vaccine, then aspartate aminotransferase activity was*

more intensively increased after a two-time vaccine administration. There was a slight decrease in calcium (by 5–9%) and phosphorus (by 2–3%) content and an increase in potassium content (by 2–5%) in the blood serum of pregnant cows two weeks after vaccine administration regardless of the sensitization scheme.

Keywords: *transfer factor, sensitization, cows, blood*

Introduction

Research and development of means for effective prevention and treatment of diseases in animals are one of the priorities for modern veterinary science. Given the low effectiveness of existing means of specific prevention of most infectious diseases of animals, in particular salmonellosis, the use of drugs based on transfer factor is relevant.

Analysis of recent researches and publications

The term "transfer factor" was proposed by H. Lawrence, who for the first time in 1955 established the possibility of transferring delayed-type hypersensitivity to tuberculin and streptococcal M-antigen in almost healthy people using donor blood lysate, sensitized to these substances (Lawrence, 1955). Transfer factors are essentially small immune messenger molecules that are produced in all higher organisms (Sell et al., 1996). Transmission factors were originally described as immune molecules that are derived from blood or spleen cells and cause delayed-type hypersensitivity and lymphokine synthesis, as well as antigen binding. They have a molecular weight of approximately 5,000 Daltons and consist exclusively of amino acids (Kirkpatrick, 1993).

The immune system helps in recognizing, fighting, remembering invading pathogens. Each pathogen can bring out a transfer factor, at least one transfer factor is created for every piece of the pathogen that the immune system faces. Transfer factors influence the activities of various immune components and also regulate cytokines. The time taken for complete development of immature immune response/delayed hypersensitivity is 10–14 days, but transfer factor induces an immune response within 24 hours (Krishnaveni, 2013). Transfer factor have many therapeutic and prophylactic applications, especially in diseases where cell-mediated immunity plays a major role (García-Hernández et al., 2014). Transfer factor has been used as a therapeutic agent in the treatment of viral, parasitic, fungal, and some bacterial infections, as well as immunodeficiencies, neoplasias, allergies, and autoimmune diseases (Lara et al., 2010; Viza et al., 2013). The main advantages of transfer factor application are efficacy for treating and also for preventing infections, low manufacturing cost, and absence of toxicity (Viza et al., 2013).

One of the stages of obtaining a qualitative transfer factor specific to a particular disease is the sensitization of the body of donor animals. The choice of donors is carried out taking into account the specific purpose of transfer factor application. Donors can be different species of animals with physiological maturity of the immune system (Fudenberg & Pizza, 1994). If the drug is prepared to prevent disease in a particular farm, it is important that the donor animals were also from this farm, herds (Petrov et al.,

1994). As an antigen to sensitize donors, standard vaccines or inactivated strains of the pathogen (viruses, bacteria, protozoa, fungi, bacterial toxins) are used. After immunization, the donor must have a high degree of sensitization of the body, which is determined by the skin reaction to the antigen to which the hypersensitivity is transferred (Galbraith & Fudenberg, 1985). The material for the preparation of transfer factor is lymphocytes obtained from lymph nodes, spleen, thoracic lymphatic duct, peripheral blood, and colostrum of sensitized donors (Rozzo & Kirkpatrick, 1992).

The purpose of the study was to investigate the blood biochemical parameters in transfer factor donor cows depending on the scheme of sensitization of their body.

Materials and methods of research

Ukrainian black-spotted dairy breed aged 4–5 years (second or third lactation) were used as donor animals to obtain the transfer factor. For the experiment, 2 groups of analogous animals were selected (10 cows per group). Sensitization of pregnant cows was carried out 1–1.5 months before calving with a concentrated formol-alum vaccine against salmonellosis (paratyphoid) of calves manufactured by the Kherson Biofactory (suspension of a formalin-inactivated culture of *Salmonella dublin* strain 373 (4 × 107 CFU/ml). The vaccine was administered to the animals of the first experimental group one month before calving, once in a dose of 10 ml. Animals of the second experimental group in 1.5 months before calving were immunized twice with an interval between injections of10 days in doses of 10 and 15 ml.

Blood samples (5 animals from each group) were taken from the jugular vein before and two weeks after the vaccine administration. The hemoglobin content was determined in the whole blood, while the activity of aspartate and alanine aminotransferases, total protein, glucose, creatinine, calcium, phosphorus, potassium, and sodium content – in blood serum.

The hemoglobin content in the whole blood was determined by the hemoglobin cyanide method – by its interaction with potassium ferricyanide, where it is oxidized to methemoglobin, which forms with acetone cyanohydrin colored hemiglobincyanide, the intensity of which is proportional to the content of hemoglobin.

Determination of aminotransferase activity (alanine aminotransferase and aspartate aminotransferase) was performed by the Reitman-Frankel method. The principle of the method is based on the fact that as a result of reamination, which takes place under the action of alanine aminotransferase and aspartate aminotransferase, oxalacetic acid and pyruvic acids are formed, which when 2.4-dinitrophenylhydrazine is added form alkaline hydrazones with an absorption maximum at wavelengths of 500–560 nm.

Determination of total protein content was performed by the Lowry method. The method combines a reaction of copper ions with the peptide bonds under alkaline conditions and a Folin–Ciocalteu reaction. The Folin-Ciocalteu reagent interacts with the cuprous ions and the side chains of tyrosine, tryptophan, and cysteine to produce a blue-green color that can be detected between 650 nm and 750 nm. The protein detection range is 5–100 μg.

Glucose content was determined by the glucose oxidase method. The principle of the method is that the oxidation

of glucose by glucose oxidase produces hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase oxidizes O-dianisidine, turning it into a compound colored blue. The color intensity is determined photometrically at a wavelength of 530 nm.

Determination of serum creatinine was performed by the Jaffe's color reaction (Popper method), where creatinine produces quantitatively an orange color with picric acid in an alkaline medium. The intensity of the color is proportional to the concentration of creatinine.

Determination of total serum calcium was performed with arsenazo III complexone. Arsenazo III is a metal complex from the group of diazo complexes that have different affinities with different metal ions. When mixing a solution of arsenazo III and serum, a calcium complex with arsenazo III is formed, which is characterized by absorption with a maximum of 650–670 nm. The absorption intensity is directly proportional to the calcium concentration in the range of 0.5–3.5 mmol/L.

Determination of inorganic phosphorus in blood serum was performed by reduction of phosphomolybdic acid. The principle of the method is based on the fact that after precipitation of proteins in the centrifuge remains inorganic phosphorus, which with molybdic acid forms phosphomolybdic acid. The latter is reduced by eiconogen to the blue phosphomolybdenum complex. The intensity of the color is directly proportional to the concentration of inorganic phosphorus in the serum.

Serum potassium concentration was determined by the turbidimetric method without deproteinization. The principle of the method is that the interaction of potassium with tetraphenylborate ions in an alkaline medium forms a stable suspension. The turbidity of the suspension, measured at a wavelength of 578 nm, is proportional to the concentration of potassium ions in the serum.

The obtained results were processed statistically. The arithmetic mean (M) and the arithmetic mean error (m) were calculated (Vlizlo et al., 2012). The probability of differences was evaluated by the probability coefficient of the Student's table (P) and the difference between the indicators was considered probable at $P < 0.05$.

Results of the research and their discussion

It is known that the sensitization of a living organism is accompanied by a restructuring of metabolism with a reorientation towards the protective systems. The conducted researches have shown a significant difference in the blood biochemical parameters in animals under different sensitization scheme (Table 1). However, it should be noted that despite the significant difference in the content of individual metabolites in the blood, the indicators did not exceed the norm, taking into account the physiological condition of pregnant cows.

Regardless of the sensitization scheme, there was a decrease in the intensity of protein metabolism in pregnant cows, resulting from a decrease in total serum protein by 2–3% and creatinine content by 13–19% ($P<0.05$–0.01) in two weeks after vaccine administration. And the simultaneous increase in the urea content in the blood serum of animals by 9–13% (although within the trend) indicates an increase in protein catabolism in the body of these animals.

The hemoglobin content in pregnant cows increased by 13% ($P<0.05$) in two weeks after re-administration

1. Blood biochemical parameters in pregnant cows under the different scheme of sensitization (M ± m, n = 5)

Period	Parameter				
	Total protein, g/L	Hemoglobin, g/L	Glucose, mmol/L	Creatinine, μmol/L	Urea, mmol/L
One-time vaccine administration					
Before sensitization	80.7 ± 1.0	91.5 ± 2.6	2 9 ± 0.1	106.0 ± 5.4	6.0 ± 0.4
14 days after sensitization	78.6 ± 4.4	93.0 ± 7.8	2.0 ± 0.2**	92.0 ± 4.7*	6.5 ± 0.2
Two-time vaccine administration					
Before sensitization	80.6 ± 1.7	91.0 ± 1.9	2.4 ± 0.1	106.5 ± 4.0	6.1 ± 0.3
14 days after the second vaccine administration	79.8 ± 1.6	102.0 ± 3.6*	2.0 ± 0.1*	86.5 ± 4.7**	6.9 ± 0.4

Note: The difference is significant at * P<0.05; ** P<0.01.

of the vaccine in comparison with the basal level. In contrast, a one-time vaccine administration did not significantly affect the hemoglobin content in the blood of animals.

Studies have shown that, regardless of the sensitization scheme, in two weeks after administration of the vaccine to pregnant cows, the blood glucose content was significantly reduced. Thus, in animals that were administered the vaccine once, the glucose content in the blood serum was reduced by 28% (P<0.01), while in pregnant cows that were vaccinated twice – by 17% (P<0.05).

The results of studies of transaminase activity in the blood serum of pregnant cows are shown in Figure 1. It was

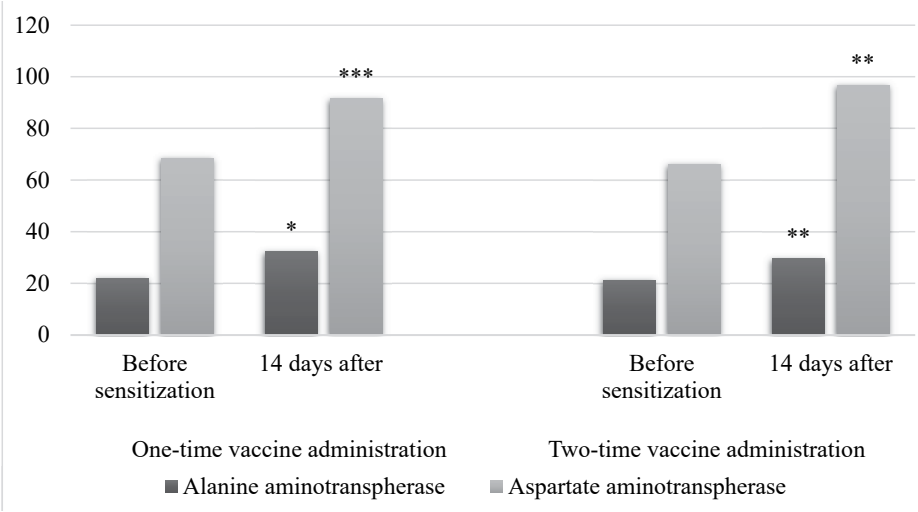

Fig. 1. The aminotransferase activity in the blood serum of cows under different sensitization scheme, units/L (M ± m, n = 5)

2. The content of macroelements in the blood serum of pregnant cows under different sensitization scheme (M ± m, n = 5)

Period	Parameter			
	Calcium, mmol/L	Phosphorus, mmol/L	Potassium, mmol/L	Sodium, mmol/L
One-time vaccine administration				
Before sensitization	2.14 ± 0.06	2.03 ± 0.03	4.05 ± 0.13	141 ± 1.29
14 days after sensitization	1.94 ± 0.11	1.99 ± 0.06	4.25 ± 0.13	142 ± 1.83
Two-time vaccine administration				
Before sensitization	2.15 ± 0.04	1.94 ± 0.04	4.03 ± 0.06	141 ± 1.29
14 days after the second vaccine administration	2.05 ± 0.04	1.89 ± 0.07	4.13 ± 0.05	140 ± 0.82

found that the activity of alanine aminotransferase and aspartate aminotransferase increased by 34–47% (P<0.05–0.001) comparing with the basal level in two weeks after sensitization depending on the vaccine administration scheme.

It should be noted that if in two weeks after one-time vaccine administration in animals, the activity of alanine aminotransferase increased more (by 46.5%; P<0.001) than aspartate aminotransferase (by 33.6%; P<0.05), then in animals which were administered vaccine twice – on the contrary, the activity of aspartate aminotransferase increased more intensively.

According to the data from Table 2, the content of individual macroelements in the blood serum of pregnant cows under different sensitization schemes did not change significantly. It is only necessary to note the insignificant decrease in the content of calcium (by 5–9%) and phosphorus (by 2–3%) and increase in the content of potassium (by 2–5%) in the blood serum of pregnant cows in two weeks after vaccine administration regardless of the sensitization scheme.

The property of most vaccines to show a suppressive effect is known, as evidenced by changes in the immunological and biochemical parameters of animals (Chen et al., 2012). Our results have shown that regardless of the sensitization scheme, there was a significant decrease in glucose and creatinine content in the blood serum of pregnant cows by 13–28% (P<0.05–0.01), a significant increase in activity of alanine aminotransferase and aspartate aminotransferase (by 34–47%; P<0.05–0.001) and a tendency to reduce the total protein content, which indicates a violation of metabolism in animals.

Conclusions and future perspectives

Thus, the sensitization of animals is accompanied by changes in metabolism that depend on the scheme of vaccine administration. An increase in the level of destructive processes in the body of pregnant cows after sensitization, accompanied by a decrease in the intensity of protein metabolism and blood glucose, an increase in the content of end products of its metabolism (urea), which may indicate an intensification of protein catabolism. However, the content of individual macro elements in the blood of pregnant cows after sensitization did not change significantly. The

suppressive effect of two-time administration of vaccine against salmonellosis of calves did not differ significantly, and in some cases was less pronounced than under one-time vaccine administration to pregnant cows.

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Анотація. *Дослідження та розроблення засобів для ефективної профілактики й терапії захворювань у тварин є одним із пріоритетних напрямів сучасної ветеринарної науки. Засоби на основі трансфер-фактора є досить перспективними для вирішення цих*

проблем. Одним з етапів отримання якісного трансфер-фактора специфічного щодо певного захворювання є сенсибілізація організму тварин-донорів. Метою даної роботи було дослідження біохімічних показників крові корів-донорів після проведення сенсибілізації за різними схемами. У дослідах використовували корів української чорно-рябої молочної породи, віком 4–5 років. Сенсибілізацію тільних корів здійснювали за 1–1,5 місяці до отелення концентрованою формол-галуневою вакциною проти сальмонельозу телят виготовленою Херсонською біофабрикою. Тваринам І дослідної групи вакцину вводили за місяць до отелення, одноразово в дозі 10 мл. Тваринам ІІ дослідної групи за 1,5 місяці до отелення вводили вакцину дворазово з інтервалом між ін'єкціями 10 діб у дозах 10 та 15 мл. Проведені дослідження показали, що в корів-донорів, яким вакцину вводили дворазово, відмічали зростання вмісту гемоглобіну на 13% (P < 0,05). Також встановлено зниження вмісту глюкози та креатиніну на 13–28% (P < 0,05–0,01) у сироватці крові тільних корів, що не залежало від схеми сенсибілізації, та тенденцію щодо зниження вмісту загального білка. Незалежно від схеми сенсибілізації корів, спостерігали підвищення активності амінотрансфераз у сироватці крові в 1,3–1,5 раза (P < 0,05–0,001), причому якщо за одноразового введення вакцини переважно зростала активність аланінамінотрансферази, то за дворазового – активність аспартатамінотрансферази. Встановлено незначне зниження вмісту Кальцію (на 5–9%) та Фосфору (на 2–3%) і збільшення вмісту Калію (на 2–5%) у сироватці крові тільних корів через два тижні після введення вакцини незалежно від схеми сенсибілізації.

Ключові слова: *трансфер-фактор, сенсибілізація, корови, кров*