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Influence of Pituitary Cytotoxic Serum on Humoral and Cellular Factors of Nonspecific Resistance of Calves' Organisms

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Abstract

This article presents information about the influence of pituitary cytotoxic serum (PCS) on the humoral and cellular factors of non-specific resistance of calves.

The Scientific research has established that under the influence of a stimulating dose there is a significant increase in humoral and cellular factors of nonspecific resistance in a month old calves. The maximum increase in the quantitative content of total protein, protein fractions, immunoglobulins, lysozyme, bactericidal, complementary activity and phagocytes was revealed on the 14th and 21st days after the administration of the drug.

Therefore, the most effective method of correction of the immune system is the administration of the biostimulator of PCS since the main effect of the drug is directed to biocorrection and bio-normalization of metabolic processes.

Keywords: pituitary cytotoxic serum, immunoglobulins, biostimulation, calf, cattle.

INTRODUCTION

The significance of the research

During the early stage of organism's growth and development, the formation of humoral and cellular factors of nonspecific resistance takes place. The occurrence and severity of many diseases in young animals are greatly influenced by various negative environmental factors that reduce the natural resistance of the organism.

Among the youngsters of farm animals, the states of immunodeficiency characterized by the reduced response to the action of various agents are often observed. They arise as a result of genetically determined congenital or acquired deficiency or defect of one or several mechanisms of the normal immune response, as well as closely related non-specific protective factors [1, 2, 3].

There are currently many ways to increase the level of nonspecific resistance of an animal organism. One of the promising areas concerning this matter is the development and effective application of the drugs that have a stimulating and immunocorrective effect on animal growth and development increasing the level of resistance of a young organism.

Application of immunostimulants makes it easier to go through post vaccinal and other types of stress conditions and the impact of negative environmental factors, improve the indices of immunity.

Despite the fact that the use of biological stimulants has been studied, many aspects of their practical application in animal husbandry require further elaboration and justification [4, 5, 6, 7].

Stimulants are considered to be the substances that enhance physiological processes, induced within the normal limits and the functional reserves potentially available in each organism.

Therefore, the search for drugs, that increase the nonspecific resistance of the body and their testing is an urgent task, especially considering calves of early age and the attempt to preserve them in good health.

Among the biological active preparations, which have a positive effect on the productive features of calves, undoubtedly high level of interest is focused on tissue-specific preparations. It should be noted that in the recent years various kinds of cytotoxic sera of directed or general stimulating action have been widely tested and introduced in production conditions [8, 9, 10, 11]. Cytotoxic serums are serums that are specific to the corresponding cells of the body. The specific antigen in them is the antigen (cell) -antibody (cytotoxin). The degree of their effectiveness depends on the intensity of the antigen-antibody reaction, which is their main mechanism of the action.

Considering cytotoxins, the matter of their mechanism of action is very important. The stimulating effect of cytotoxins is explained by the fact that in small doses they produce the same small disruptions in homologous organs, that are very sharply manifested in large doses. But at low doses, these disruptions occur correspondingly in smaller numbers with the predominant lesion of pathologically altered cells.

Products of degradation of protein are physiological stimulators of vital activity and are capable of activating the basic functions of cells-nutrition, growth and multiplication, as well as changing the reactivity of cells and tissues to the action of a number of substances [12, 13, 14, 15].

Studies confirmed the theory of the mechanism of action of cytotoxins, suggested by A. A. Bogomolets, according to whom, under the influence of cytotoxic sera in the corresponding organ, a reaction of the combination of cytotoxic serum antibodies with the antigen of the cell or organ takes place [16]. In this case, a reverse anaphylaxis reaction occurs and then the antigen-antibody complex decomposes under the action of cellular enzymes. The products of this disintegration are specific substances which, depending on the dose, cause either stimulation of the cell function or their inhibition [17].

Such immunostimulants might be represented by pituitary cytotoxic serum (PCS) obtained by hyperimmunization of pituitary tissues of animals intended for slaughter.

The aim and objectives of the research

The aim of the article is to study the effect of the stimulating dose of "PSC" on the humoral and cellular immune protection factors of one-month-old calves.

The following research tasks were set:

1. Study of the effect of the stimulating dose of "PSC" on the dynamics of the total protein and protein fraction of blood serum of calves;
2. Study of the effect of the stimulating dose of "PSC" on the dynamics of lysozyme, bactericidal and complementary activity of the calf serum;
3. Study of the effect of the stimulating dose of "PSC" on the dynamics of the immunoglobulin composition of the calf serum;
4. Study of the effect of the stimulating dose of "PSC" on the dynamics of the indices of the cell link protecting the calves.

MATERIALS AND METHODS

The study was performed on 12-month-old calves of the Alatau cattle breed originated from a farm located in the Almaty region. The calves were of both sexes and similar weight (approx. 26-28 kg). The animals were kept in the same conditions and were

fed the same feed. The calves were randomly divided into two groups (experimental and control), consisting of 6 animals each.

The animals were regularly subjected to veterinary examination and planned preventive treatments. The experimental group was administered a challenging dose (0.1 ml3 per kg of bodyweight) and the control group was administered not immune (native) serum.

The drug was made in the laboratory of the Department "Clinical veterinary medicine" of KazNAU according to the method developed by the Department.

The blood for the analyses was sampled from the jugular vein in the following sequence: before the administration of the drug (background data) and after administration on 7^{th}, 14^{th}, 21^{st} and 28^{th} day.

Laboratory blood tests were carried out in the clinical diagnostic laboratory of the medical center "Sana" (Almaty). The quantitative values of immunoglobulins, lysozyme, bactericidal and complementary activity were determined using the Reader devices StatFax 2100IFA, (USA); ELISA autoimmune analyzer Immulite 1000 (USA), phagocytic activity using MBL 2000 microscopes, Carl Zeiss, Prima Star (Germany); M-50, (Austria); total protein-refractometric; protein fractions, by horizontal electrophoresis on an agar gel.

The obtained digital data was processed by the constant method of variational statistics with the calculation of the arithmetic mean values and their statistical errors (Mean ± SEM), the reliability (P) of the compared indicators was determined using the student's T-test. Statistical analyses were performed using the Statistica software.

RESULTS AND DISCUSSION

One of the main indicators that reflect the general state of animal organisms is the change in the protein composition of the blood. Total protein concentration and concentration of protein fractions are very important parameters that indicate the vital activity and can be used for estimation of the effectiveness of therapeutic and bio-stimulating agents.

As a result of the experiments, we have obtained evidence of the favorable effect of the PSC on the protein pattern of blood serum of the calves. These changes affect the content of both the total protein and its individual fractions (Table 1). From the data of Table 1, it can be seen that before the administration of the drug, both the total protein and its individual fractions were approximately at the same level. The changes begin to manifest themselves in the subsequent periods of research, especially under the influence of PSC in the experimental group of calves.

A significant increase in the total protein concentration and its fractions was observed in experimental animals in comparison to the control group. On the 7th day after the two-fold injection of the drug an increase in the level of total protein was observed, and compared to the initial index, the rate of increase in one-month calves was 3.0%.

On the 14^{th} day, the crude protein content began to increase, wherein the test indicator value was increased by 9.2% compared to the baseline data ($P<0.05$).

In the subsequent terms of the study (21^{st} and 28^{th} days) the level of crude protein in the blood serum of the calves compared to the previous period tended to decrease, but compared to the initial index, the increase was 6.5 and 3.5% ($P< 0,01$). In the control group, the crude protein content almost did not change and was within 67.02±1.23-68.07±1.40g/l.

Table 1 – Effect of PCS on the dynamics of the protein composition of blood serum of one-month calves, in g / l, (Mean ± SEM, n =10).

Indicator	Group	Before PSC injection (background)	Days after the drug administration			
			7	14	21	28
Crude protein	Experimental	68.77 ± 1.48^{x}	70.81 ± 1.12^{x}	75.12 ± 1.23^{xx}	73.23 ± 0.98^{xx}	71.18 ± 1.11^{x}
	Control	68.07 ± 1.40^{x}	67.04 ± 1.46	67.48 ± 1.14^{x}	67.14 ± 1.16^{x}	67.02 ± 1.23
PROTEIN FRACTIONS:						
Albumins	Experimental	31.65 ± 0.14^{x}	32.94 ± 0.10^{x}	33.35 ± 0.08^{xx}	35.18 ± 0.08^{xxx}	33.97 ± 0.09^{x}
	Control	31.93 ± 0.09^{xx}	31.89 ± 0.10	31.11 ± 0.11^{x}	32.21 ± 0.10^{x}	$31,37 \pm 0.08$
Globulins	Experimental	37.12 ± 0.31^{x}	37.87 ± 0.21	41.77 ± 0.29^{xx}	38.05 ± 0.25	37.21 ± 0.28^{xxx}
	Control	36.14 ± 0.41	35.15 ± 0.32	36.37 ± 0.30^{x}	34.93 ± 0.22^{x}	35.65 ± 0.28
α-globulins	Experimental	9.96 ± 0.09^{x}	9.48 ± 0.07	10.61 ± 0.09^{x}	10.79 ± 0.07^{xx}	10.25 ± 0.08
	Control	9.27 ± 0.13	9.29 ± 0.07	9.83 ± 0.09^{x}	9.94 ± 0.06^{x}	10.15 ± 0.08^{x}
β- globulins	Experimental	10.92 ± 0.07^{xx}	10.87 ± 0.05	9.51 ± 0.08^{xx}	$7,95 \pm 0.05$	8.94 ± 0.08
	Control	10.10 ± 0.13^{x}	10.05 ± 0.09	9.67 ± 0.08^{x}	7.25 ± 0.05	8.81 ± 0.07
γ - globulins	Experimental	16.24 ± 0.14^{x}	17.52 ± 0.10	21.65 ± 0.12^{xxx}	19.31 ± 0.13^{xxx}	18.02 ± 0.12^{xx}
	Control	16.77 ± 0.15	15.81 ± 0.16^{x}	16.87 ± 0.13^{x}	17.74 ± 0.11^{x}	16.69 ± 0.12
A/G coefficient	Experimental	0.853^{x}	0.870^{x}	0.798	0.925^{xx}	0.913^{x}
	Control	0.884	0.907	0.855^{x}	0.922	0.880

Date: $^{x}P < 0.05$; $^{xx}P < 0.01$; $^{xxx}P < 0.001$ – reliability of the received data: concerning the beginning of the experiment and in comparison with the control group; A/G-albumin-globulin coefficient.

Notes: ($P< 0.05$).

Table 2 – Influence of PSC on indicators of humoral protection of the organism of one-month-old calves, (Mean ± SEM, n=30), %.

Indicator	Group	Day of the research				
		background data	7	14	21	28
LZ	E	55.6±0.56	$57.2±0.58^{xx}$	$60.4±0.96^{xxx}$	$61.5±0.81^{xx}$	60.4±0.69
	C	55.2±0.61	$55.6±0.80^{x}$	$55.8±0.71^{x}$	$56.9±0.80^{xx}$	56.5±0.74
MB	E	38.1±0.32	$40.1±0.24^{xx}$	$43.8±0.49^{x}$	$44.1±0.36^{xxx}$	43.9±0.57
	C	38.4±0.39	$38.9±0.30^{x}$	$39.2±0.22^{x}$	$39.4±0.51^{x}$	39.3±0.38
CA	E	15.2±0.29	$16.9±0.38^{xx}$	$18.6±0.31^{xxx}$	$19.8±0.61^{x}$	18.5±0.77
	C	15.3±0.28	15.8±0.33	$15.9±0.24^{x}$	$15.8±0.34^{x}$	15.7±0.56

Note: LZ- lysozymic, MB- microbicide, CA- complement activity;
E-experimental group, C-control group; $^{x}P<0.05$; $^{xx}P<0.01$; $^{xxx}P<0.001$ ^{x}relative to the beginning of the experiment and in comparison with the control group.

It was found that the preparation has a pronounced stimulating effect on the dynamic of the protein content of the individual fractions. From the results of the studies, it can be seen that under the influence of the drug, the albumins tended to increase during all periods of research. On the 7th day after injection of the drug, the level of albumins increases by 4.1% compared to the initial index; on the 14th day - 5.4%, on the 21st day -12.2%; on the 28th day - 7.3% (P<0.05; P<0.01; P<0.001).

In the compared control group, the content of albumins did not undergo any special changes.

The administration of the drug had a certain effect on globulin fractions of the protein. In the experimental group, the increase in globulin fractions on the 14th and 21st days after administration of the drug relative to the baseline was 12.5% and 2.5%, respectively and in the control group the indices tended to decrease slightly. β- globulin fractions of proteins of the calves tended to decrease both in the experimental and control groups. Despite the decrease in quantitative ratio, the value of this fraction in one-month calves of the experimental group on days 21 and 28 was 9.6% and 1.5%, significantly higher than in the control group of animals.

α - globulins, in contrast to the β-globulin fractions in the experimental group of calves, tended to increase; on the 14th, 21st and 28th days after the administration of the drug, relative to the background level, it increased by 6.5%, 8.3% and 2.9% respectively and in the control group the degree of increase was insignificant (P < 0.01; P < 0.05).

The administration of the drug had a significant effect on the content of γ-globulin fractions of the protein, which are protective proteins. The level of this fraction after administration of the drug in all periods of the study compared with the control group was significantly increased. Even on the 7th day of the study, the level of γ-globulins had already increased by 7.9% in one-month calves; on day 14 it reached the highest level with the increase of 33.3%, and on the 21st and 28th day of the study the degree of the increase was 18.3% and 11.1%, respectively, compared to the baseline (P< 0.01; P < 0.05; P < 0.001). In the control group, the degree of the increase with respect to the experimental group was insignificant and made up from 16.77 ± 0.15 – 17.74 ± 0.11 g/l (P< 0.05).

The administration of PSC causes favorable changes not only in the total protein and protein fractions concentrations but also positively affects the albumin-globulin (A/G) coefficient.

Thus, the A/F coefficient in the experimental group of one-month calves on the 21st day of the study increased by 8.4% and by the end of the experiment by 7%. In the control groups of animals, the studied parameters did not change significantly and remained approximately at the same level during all periods of the study with insignificant fluctuations.

Based on the above stated, it should be noted that the drug has a stimulating effect on the overall protein pattern and protein fraction of the blood serum of calves. Therefore, the main effect of the drug is aimed at biocorrection metabolic processes in the organs and tissues of the calves, which is confirmed by the significant increase in the level of total protein and protein fractions.

Today the phenomenon of natural immunity is considered as a result of the combined activity of humoral and cellular factors that ensure the stability of the immune-structural homeostasis of the internal environment with all kinds of adverse effects.

Numerous studies have shown that proteins are the most important factors of humoral protection that reflect the potential ability of the body to mobilize protective forces and therefore the determination of their activity in the study of natural immunity is of paramount importance [18, 19, 20].

The results of the conducted study show that before the introduction of PSC the initial data in the calves for the analyzed parameters in both experimental and control groups of animals did not differ significantly (Table 2).

In subsequent periods of research, we found that the indices of humoral immunity of the calves in the control group were below the physiological norm and significantly lower than in the experimental group of calves, what can indicate an immunodeficiency state.

The administration of the PSC stimulator significantly activates the lysozyme, bactericidal and complementary activity of the calf serum (LAS, BAS, CAS). After the treatment of the calves for 7 days, LAS levels increased by 2.9%, after 14 days by 8.6% compared to the data before the administration and reached the maximum value on the 21st day of the study - 10.6% (P<0.05, P<0.001). The LAS timing control animals varied slightly above and ranged from 55.2 ± 0.61 to 56.9 ± 0.80% (P<0.05).

The administration of the stimulating dose of the drug to one-month-old calves causes certain changes in the dynamics of BAS. The degree of increase in ALS compared to the background indicator is as follows: on the 7th day the increase was 5.2%; on the 14th day -15.0%; on the 21st day - 15.7% and on the 28th day - 15.2% (P<0.05; P<0.01).

The level of BAS in the specified periods in the control group of calves also tended to increase, but the indices were significantly lower than those of the experimental group of animals.

The level of CAS under the influence of PCS also significantly increased. On the above days, the CAS indicator increases in respect to the background level, respectively, by 11.2%; 22.4%; 30.3% and 21.7%. The CAS values in the control group range from 15.3 ± 0.28-15.9 ± 0.24% (P<0.05).

One of the components of humoral immunity is immunoglobulins. Determination of the quantitative composition of immunoglobulins makes it possible to determine the state of the natural resistance of the calves' organism. It should be noted that the content of immunoglobulins in blood serum of all age groups was dynamic. More pronounced changes were observed in the experimental group of calves (Table 3).

Table 3 – Impact of PSC on the composition of serum immunoglobulin one-month calves, in mg/ml, (Mean ± SEM, n = 30).

Immunoglobulins	Groups	Before injection of PCS	After PCS injection (days)			
			7	14	21	28
IgG	E	16.8±0.21	18.4±0.18^{x}	21.1±0.22^{xx}	21.8±0.19^{xxx}	20.6±0.21^{x}
	C	16.9±0.29	17.1±0.21	17.4±0.26	17.2±0.32^{x}	17.3±0.29
IgM	E	1.46±0.12	1.64±0.11	1.82±0.12^{xx}	2.01±0.18^{xxx}	1.94±0.08^{xxx}
	C	1.47±0.14	1.52±0.18	1.66±0.09	1.72±0.11	1.73±0.16
IgA	E	0.36±0.,08	0.51±0.07^{x}	0.62±0.08^{xxx}	0.64±0.09^{x}	0.61±0.09^{xx}
	C	0.37±0.09	0.40±0.08	0.42±0.07^{x}	0.41±0.08	0.40±0.07
Total Ig	E	18.6±0.51	20.6±0.46^{xx}	23.4±0.52^{x}	24.5±0.46^{xx}	23.5±.48^{xx}
	C	18.7±0.62	19.0±0.47	19.5±0.52	19.3±0.51^{x}	19.4±0.32

Note: IgG-G immunoglobulins ; IgM-M immunoglobulins ; IgA- A immunoglobulins; E- experimental, C-control group; ^{x} P<0.05; $^{x\,x}$ P<0.01; $^{x\,x\,x}$ P<0.001 ^{x} relative to start of the experiment and compared with the control group.

Table 4 – Impact of PSC on dynamics of the index of cellular immunity of one-month-old calves, (M±m, n=10).

Indicators		Group	Before injection of the drug	After drug injection (days)		
				7	14	21
PhAL according to NST-test,%	Spontaneous phagocytosis	E	19.4±0.25^{x}	23.7±0.17^{x}	24.2±0.19^{xxx}	23.9±0.18^{xx}
		C	19.7±0.19	20.2±0.19^{x}	20.6±0.15^{xx}	20.5±0.16^{xx}
	Induced phagocytosiswith LPS	E	43.9±0.25	49.8±0.27^{xx}	47.8±0.36^{xx}	44.2±0.26^{xx}
		C	43.8±0.31^{x}	44.5 ±0.24	44.3±0.27	43.9±0.32^{x}
Phagocytosis,%	Spontaneous phagocytosis	E	17.6±0.15^{x}	20.6±0.17^{xx}	24.5±0.11^{xx}	22.6±0.19
		C	17.4±0.11	18.8±0.18^{x}	19.5±0.19^{x}	19.1±0.19^{x}
	Induced phagocytosiswith LPS	E	41.5±0.12^{x}	46.2±0.27^{x}	47.9±0.18^{xxx}	44.2±0.24
		C	41.7±0.28	42.9±0.35^{x}	43.2±0.20^{x}	42.0±0.25

Note: ^{x}P<0,05; ^{xx}P<0,01; ^{xx}P<0,001-reliability; E-experimental group; C-control group;
PhAL- phagocytic activity of leukocytes; NST- nitrosine tetrazole; LPS- lipopolysaccharide.

In the process of analyzing the immunoglobulin composition of the blood serum, we found out that its quantitative content in the experimental group during all study periods was significantly higher than in the control group of animals. Thus, the level of IgG after the administration of the drug gradually increased and reached its highest value on the 14th, 21st and 28th days of the study.

The increase in the concentration of IgG in the one-month calves of the experimental group in the indicated periods compared with the initial data was 25.6%; 29.8% and 22.6% (P<0.05; P<0.01). But in the control group, no significant changes were registered.

The similar trend was observed for IgM. Under the influence of PCS, the IgM content in the above-mentioned periods significantly increased by -24.7%, respectively; 37.7% and 32.9%(P<0.05).

In the control group the amount of IgM was without significant changes and ranged between 1.47 ± 0.14 - 1.73 ± 0.16 mg/ml (P<0.05).

The maximum increase in Ig A in the experimental group was observed on the 14th and 21st days of the study from 0.36±0.08, respectively, to 0.62±0.08 и 0.64±0.09 mg/ml (P<0.01, P<0.05; P<0.001). In the control group, significant changes did not occur.

The number of immunoglobulins in the experimental group was significantly higher than the control group in all the study periods, and the increase was 10.8% on the 7th day; on the 14th day - 25.8%; on day 21st - 31.7%; on the 28th day - 26.3% (P < 0.01 – 0.05).

So, it can be stated based on the study of the humoral immunity factor that PCS has a pronounced corrective and stimulating effect on the protein index, lysozyme, bactericidal and complementary activity and immunoglobulin composition of the calf serum.

One of the main criteria for assessing the immunobiological state of a body of a young animal is the study of the indices of the cellular link in the protection of immunity. Phagocytosis of leukocytes is a powerful barrier to the penetration and spread of microbes in the body. It is one of the factors of both natural congenital and specific acquired immunity. The degree of leukocyte phagocytosis must be one of the important indicators of the immunobiological state and is of great importance in determining the level of natural resistance of animals [4].

It should be noted that the stability of young animals, especially from an early age to various kinds of diseases largely depends on the level of indicators of cellular immunity of their organisms.

The Research has established that the PCS has a stimulating effect on cellular factors calves immunity. The determination of phagocytic activity was carried out in the clinical diagnostic laboratory of the MC "Sana" by two methods

1. NST-test: a) spontaneous phagocytosis, b) induced phagocytosis with PCS; 2- phagocytosis with latex.

Information about the impact of PCS on the cell link of the immunity of calves is presented in Table 2.

In Table 4 the impact of PSC on cellular immunity was presented. The difference in rates comes after the introduction of PCS.

Phagocytic activity of blood NST-test: spontaneous phagocytosis on the 7th and 14th days after the injection of the drug relative to the initial data increased by 22.2% and 24.7%, respectively, phagocytosis induced with LPS by 13.4% and 8.9%.

Spontaneous phagocytosis increased by 17.0% and 39.2%, phagocytosis induced with LPS by 11.3% and 15.4% (^{x} P<0.05; ^{xx} P<0.01; ^{xx} P<0.001). At the specified time the control group to the special unaltered and remained at approximately at the same level.

By the end of the experiment, i.e. on day 21 after the administration of PCS leukocyte phagocytic activity decreases slightly, but nevertheless in comparison with the control group of calves remained fairly high. According to the NST-test: spontaneous phagocytosis-decreased 15.4%; induced phagocytosis with LPS-2.3%; phagocytosis with spontaneous latex-18.3%, induced with LPS- 5.2% (^{x} P<0.05; ^{xx} P<0.01).

The changes that occurred in the parameters of the control group of calves were connected to their age. However, the degree of improvement was much lower than in the experimental group. Thus, on the 30th day after the administration of PSC, the phagocytic activity of leukocytes increased. According to the NST-test: spontaneous phagocytosis increased by 12.9%, phagocytosis induced with LPS by 3.4%, spontaneous phagocytosis with a spontaneous increase of 8.1%, phagocytosis induced with LPS by 2.9%. On the 60th day after the PSC administration, the phagocytic activity of the blood increased according to the NST test: spontaneous phagocytosis increased by 14.4%, phagocytosis induced with LPS by 6.8%; phagocytosis with latex spontaneous increased by 12.1%, phagocytosis induced with LPS by 6.0%. We can conclude, that the studied serum stimulates the phagocytic activity of blood cells, and that play a decisive role in the system of natural resistance and immunity of the body.

CONCLUSIONS

Thus, we can conclude based on the analysis of the presented data that calves of early age often have an immunodeficient state accompanied by an imbalance of the immune system. Therefore, the most effective method of correction of the immune system is the use of a biostimulator of PCS, since the main effect of the drug is directed at biocorrection and bio-normalization of metabolic processes. Our study also showed that young calves have lower rates of cellular immunity and therefore require the administration of drugs that correct immunity imbalance. We are confident that the studied drug used meets those requirements.

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