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Influence Of Phytopreparations Based On *Hypericum perforatum* On The Formation Of Natural Resistance Of Calves And Sheep.

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ABSTRACT

The aim of the research was to study the level of influence of phytopreparations obtained on the basis of *Hypericum perforatum* by the traditional method and by using ultrasound on the indices of natural resistance of the organism of calves and sheep spontaneously infested with strongylates. As a result of a comprehensive research a high degree of their pharmacological influence on the parameters of cellular and humoral immunity was determined.

Keywords: phytopreparations, *Hypericum perforatum*, sheep, calves, natural resistance, dry extract, ultrasound.

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INTRODUCTION

Successful development of animal husbandry depends in many ways on the state of the fodder base used in animal feeding. Wherein, the most common and cheap is fodder of vegetable origin, which includes herbs of natural meadows and pastures, as well as sowing forage crops. Forage plant cultures contain in their composition nutrients and biologically active substances necessary for the normal functioning of the animal's organism and obtaining from them a full agricultural product. Many plants, which contain in fodder diets, take an immediate part in metabolic processes and some of them participate in physiological stimulation of the organism, affecting the natural resistance of animals, increasing immunity and resistance to stress [1,3,12,13]. Medicinal plants actively help to fight with helminthes and skin parasites in animals [8]. These plants are also irreplaceable in violation of digestion. Moreover, they are used both in disorders, including intestinal infections, and with costiveness associated with improper feeding and "hypodynamic" way of life of pets.

The use of such plants or phytopreparations prepared from them can become one of the priority directions of pharmacological science aimed at increasing the natural resistance, safety and productivity of animals [2,4,5,7,10].

Thus, studying the pharmacokinetics of preparations obtained on the basis of *Hypericum perforatum* (tincture, liquid and dry extracts), we observed a tendency to increase the indices of natural resistance of the animal organism [6,9,11]. Moreover, a direct relationship between the content of flavonoids in the preparation and the level of natural resistance of the animal organism was determined [1,14].

The aim of our research was to study the influence of phytopreparations obtained on the basis of *Hypericum perforatum* by using ultrasound and by the traditional method with enteral injection on the indicators of natural resistance of the organism of calves and sheep spontaneously infested with strongylates.

METHODOLOGY OF RESEARCH

As the materials for study we used the preparations obtained on the basis of *Hypericum perforatum*. In the experiment we used tincture, liquid and dry extract of *Hypericum perforatum*, as well as dry extract of *Hypericum perforatum*, processed by ultrasound.

Tincture of *Hypericum perforatum* (1:5) was prepared on the basis of 40° alcohol by method of maceration. *Hypericum perforatum* liquid extract was a concentrated extract from the herb of *Hypericum perforatum* with ethyl alcohol in a ratio of 1:1, obtained by percolation. Dry extract of *Hypericum perforatum* was obtained by drying the liquid extract in a vacuum oven with a moisture content of not more than 5%. The dry extract after sonication was a 0.1% aqueous suspension with particle sizes of the extract of 2-20 µm.

The study of influence of the preparations of *Hypericum perforatum* on the indices of natural resistance of the animal organism was carried out on sheep at the age of 11-12 months and on calves at the age of 9-10 months. For the study, animals spontaneously infested with strongylates were used. To set up the experiment, groups of animals were formed according to the principle of paired analogs of 10 animals in each, taking into account the schemes of application of the preparations.

In the first series of experiments the efficiency of *Hypericum perforatum* preparations was evaluated by their effect on the sheep organism. The animals of the first four groups were experimental, they received preparations of *Hypericum perforatum* enterally according to the following scheme: 1st group received the dry extract, processed by ultrasound, once in a dose of 5 mg/kg; 2nd group received the dry extract, processed by ultrasound, once in a dose of 10mg/kg; 3rd group received the liquid extract of *Hypericum perforatum* twice in a dose of 0.1 ml/kg of body; 4th group received the dry extract of *Hypericum perforatum* twice in a dose of 15 mg/kg. Sheep of the 5th group received a 2.5% suspension of albazene (base preparation). Animals of the 6th group served as biological control (without using preparations).

The second series of experimental studies was carried out on calves. The first four groups of calves enterally received preparations of *Hypericum perforatum* according to the following scheme: 1st group received the liquid extract of *Hypericum perforatum* twice in a dose of 0.1 ml/kg; 2nd group received the dry extract, processed by ultrasound, once in a dose of 10 mg/kg; 3rd group received 2.5% suspension of albazene

in a dose of 3 ml/40 kg; 4th group received the dry extract of *Hypericum perforatum* twice in a dose of 15 mg/kg. Calves of the 5th group served as control, without getting any preparations.

Blood for the research was selected from the jugular vein in dynamics – before treatment with preparations and then after one, three, seven and fourteen days of the experimental period. From the indicators of natural resistance, the bactericidal and lysozyme activity of blood serum was determined, as well as the indices of phagocytosis according to the "Methodological Guidelines for Determining Natural Resistance and Ways to Increase it in Young Farm Animals" [6]. Statistical processing of the results was carried out using special software packages. The study of quantitative characteristics was carried out by comparing the mean values of two sample sets with the definition of the Student's test and the level of significance (p).

RESULTS

When studying the indices of natural resistance of sheep and calves, the following results were obtained.

The study of lysozyme activity of blood serum (LABS) of sheep in control and experimental groups significantly showed that the use of preparation forms of *Hypericum perforatum* stimulates the increase of this index (Table 1). Already on the third day of the use of preparations in animals of the 1st and 2nd test groups LABS exceeded the control analogues by 2.95% and 5.92%.

Table 1: Influence of preparative forms of *Hypericum perforatum* on indices of natural resistance of the organism of sheep (M±m; n=10)

Groups of animals	Before using preparative forms	After using preparative forms, days				
		1	3	5	10	14
Lysozyme activity of blood serum, %						
1 experimental	16.80±0.33	16.90±0.30	17.40±0.25	16.40±0.4	17.0±0.6	16.90±0.30
2 experimental	17.40±0.60	16.80±0.30	17.90±0.27	16.60±0.3	17.0±0.29	17.20±0.10
3 experimental	16.70±0.30	16.40±0.10	16.40±0.08	16.40±0.2	15.8±0.24	16.80±0.20
4 experimental	17.00±0.23	16.50±0.10	16.40±0.14	16.80±0.3	16.8±0.23	16.90±0.40
5 experimental	16.80±0.39	14.5±0.3**	15.50±0.41	16.50±0.4	17.1±0.30	16.50±0.10
6 control	16.80±0.34	16.90±0.10	16.90±0.34	16.70±0.2	16.7±0.33	16.90±0.20
Bactericidal activity of blood serum, %						
1 experimental	60±15.6**	73.80±7.60	72.6±7.10	83.9±6.2*	79.8±4.60	84.5±3.20*
2 experimental	50±20.8**	65.9±7.2**	79.7±6.60	75.0±4.70	81.1±3.80	78.20±3.90
3 experimental	78.60±4.53	74.50±6.20	77.0±7.5*	68.7±6.00	84.1±4.70	76.20±3.30
4 experimental	79.60±6.30	60±5.8***	76.9±5.8*	67.0±7.90	76.5±8.40	75.80±5.50
5 experimental	69±11.5**	59±12.5***	81.40±7.48	79.5±6.8*	84.7±4.53	80.40±3.80
6 control	84.5±4.65	79.70±4.10	87.2±3.94	68.5±9.40	79.6±4.90	72.20±5.80

Note: * – P<0,05; ** – P<0,01; *** – P<0,001

In other groups this indicator remained within the norm, although it was lower than in the control group and there were no significant differences between the animals of the experimental and control groups.

Animals of the same groups also had the highest bactericidal activity of blood serum (BABS). The trend towards its increase was noted starting from the fifth day of the experiment in all groups where *Hypericum perforatum* preparations were used, especially in groups with the use of a dry extract, processed by ultrasound. The increase in bactericidal activity in the 1st and 2nd experimental groups on the third day of the experiment relative to background indicators was 21.0% and 59.4%. On the fifth day of the study the indicator of BABS in these groups exceeded the control indicators by 22.4% and 9.5%, and on the 14 day of the experiment exceeded them by 17.0% and 8.3% respectively.

As a result of the experiment on sheep it was found out that the dry extract of *Hypericum perforatum*, processed by ultrasound, in a dose of 10 mg/kg shows the activation of unspecific protection of the sheep

organism – BABS and LABS. These data were the basis for conducting experiments in the conditions of production on calves (Table 2).

Table 2: Influence of preparative forms of Hypericum perforatum on indices of natural resistance of the organism of calves (M±m; n=10)

Groups of animals	Before using preparative forms	After using preparative forms, days			
		1	3	7	14
Lysozyme activity of blood serum, %					
1 experimental	24.46±0.41*	25.40±0.29*	18.76±4.70	23.08±0.80	24.94±0.34
2 experimental	23.46±0.42	24.50±0.12	18.44±4.62	23.30±0.34	25.00±0.11*
3 experimental	25.14±0.58*	26.02±0.40*	20.76±5.20	26.14±0.7*	25.62±0.22*
4 experimental	24.25±0.57	24.40±0.55	18.42±6.14	24.4±0.24*	24.42±0.34*
5 control	19.54±4.88	19.70±4.93	14.96±6.10	19.70±4.93	19.98±4.99
Bactericidal activity of blood serum, %					
1 experimental	30.5±9.28***	51.3±9.47**	47.02±14.00*	58.2±7.1**	57.83±14.61
2 experimental	49.3±12.20	38.60±4.12	43.24±14.90	34.0±14.45	57.82±15.39
3 experimental	43.46±2.65	35.30±10.20	38.39±11.40	50.2±11.4*	44.87±11.88**
4 experimental	34.73±9.80**	22.60±8.58*	39.99±17.20	53.0±16.49	39.9±15.55***
5 experimental	48.69±5.27	33.30±11.10	33.40±19.05	43.70±11.5	50.96±5.50

Note: *– P<0.05; **– P<0.01

The data of Table 2 show that the LABS throughout the experiment in all the calves of the experimental groups was higher than in the control animals. In the 2nd experimental group the maximum increase in lysozyme activity of blood serum relative to background values was noted. In comparison with the control, this indicator in the group on the 14 day exceeded the control values by 25.12% (P <0.01). The indicator of BABS in the 2nd experimental group on the 14 day of the experiment increased relative to the control analogs by 13.46%.

Along with the humoral factors of immunity, the cellular factors were also studied such as the phagocytic activity of neutrophils, the phagocytic index and the phagocytic number, which are one of the main indicators of the immunological restructuring of the organism (Table 3). It should be noted that in the calves of the second experimental group the phagocytic activity of neutrophils on the 14 day of the experiment was higher by 13.0% than the control values, the increase in the phagocytic index and the phagocytic number was manifested at the trend level.

Table 3: Influence of preparative forms of Hypericum perforatum on indices of phagocytosis of calves (M±m; n=10)

Groups of animals	Before using preparative forms	After using preparative forms, days			
		1	3	7	14
Phagocytic activity of neutrophils, %					
1 experimental	45.8±1.37*	43.20±0.69	35.28±8.83	45.0±0.68*	43.08±0.56
2 experimental	43.46±0.60	41.50±0.31	37.74±9.45*	45.3±1.20*	45.50±0.53
3 experimental	43.60±0.90	43.30±1.15	37.02±9.27*	43.50±0.40	44.52±1.44
4 experimental	45.8±0.64*	46.10±1.42*	31.42±10.40	45.5±0.90*	44.35±1.00
5 control	37.50±9.50	37.70±9.48	27.50±11.24	35.70±8.94	36.26±9.08
Phagocytic index					
1 experimental	2.27±0.02	2.30±0.04*	2.32±0.04	2.36±0.03	2.26±0.08
2 experimental	2.29±0.01	2.38±0.03	2.32±0.03	2.37±0.03	2.38±0.03
3 experimental	2.29±0.02	2.4±0.02	2.35±0.04	2.34±0.03	2.33±0.04

4 experimental	2.31±0.01	2.38±0.03	2.33±0.04	2.35±0.04	2.34±0.06
5 control	2.28±0.08	2.33±0.03	2.36±0.03	2.33±0.04	2.34±0.04
Phagocytic number					
1 experimental	0.96±0.008	0.97±0.01	1.06±0.009	1.04±0.01	1.05±0.02
2 experimental	0.95±0,01	0.95±0.01	1.05±0.01	1.05±0.01	1.04±0.01
3 experimental	0.94±0.01	0.94±0.01	0.95±0.01	0.95±0.01	0,954±0.01
4 experimental	0.95±0.01	0.96±0.01	1.08±0.01	1.07±0.02	1.05±0.009
5 control	0.93±0.006	0.9±0.006	0.96±0.01	0.93±0.01	0.92±0.01

Note: *– P<0.05; **– P<0.01; ***– P<0.001.

In determining the anthelmintic activity of *Hypericum perforatum* preparations in the treatment of strongylatoses of the gastrointestinal tract of calves and sheep, a 90% extensivity was determined in the groups where the preparation was processed by ultrasound. After exposure to ultrasonic waves anthelmintic properties of phytopreparations improved and their therapeutic effect and antiparasitic activity increased.

CONCLUSION

Thus, it has been determined that phytopreparations obtained on the basis of *Hypericum perforatum* make it possible to activate non-specific humoral and cellular immunity – lysozyme and bactericidal activity of blood serum and phagocytosis of calves and sheep spontaneously infested with strongylates. In this case, a dry extract processed by ultrasound has a particularly effective action. Therefore, preparations obtained on the basis of *Hypericum perforatum*, in particular dry extract, processed by ultrasound, can be used as natural immunostimulants of young farm animals.

REFERENCES

- [1] Avdachenok VD. Toxicopharmacological characteristics of the preparation forms of *Hypericum perforatum* and their effectiveness in the strongylatoses of the gastrointestinal tract of sheep and calves: Abstract of the PhD thesis (Vet. Sci.): 03.00.19, 16.00.04. Vitebsk, 2006.
- [2] Abramov SS, Mogilenko AF, Yatusevich AI. Methodical guidelines for determining the natural resistance and ways of its increase in young farm animals. Vitebsk, 1989: 40.
- [3] Gorshkov GI, Yakovleva EG. Is there an alternative to antibiotics? *Veterinary Bulletin*. 2013; 8 (138): 6-8.
- [4] Smirnov PN. Study of the adaptive capabilities of agricultural animals in Siberia: monograph. Novosibirsk Agrarian University, Novosibirsk, 2006; 195.
- [5] Madonov PG, Kinsht DN. New possibilities for improving the pharmacokinetic properties of medicines. Proceedings of the XII International Scientific Congress "Rational Pharmacotherapy". St. Petersburg, 2017; 96-98.
- [6] Maksimov VI, Verhovskiy OA, Moskvina AS. Change in the picture of blood of cattle during vaccination. *Veterinary, zootechny and biotechnology*. 2014; 8: 40-48.
- [7] Maksimov VI, Parakhnevich AV, Parakhnevich AA, Glagoleva TI, Kutafina NV. Physiological reaction of erythrocytes' micro rheological peculiarities in milk fed piglets after the negative impact of the environment. *Annual research & review in biology*. 2017; 17 (1): 1-8.
- [8] Nozdrin GA et al. Scientific basis for the use of probiotics in poultry farming: monograph. Novosibirsk Agrarian University, Novosibirsk, 2005; 23.
- [9] Nikonova EB, Maksimov VI. Suppression of T-lymphocytes in mink on the background of the disturbance of mineral metabolism. *Veterinary pathology*. 2006; 3: 128-132.
- [10] Tarasov MB, Gorshkov GI. Investigation of nanostructured preparations with given properties. *Nano- and supramolecular chemistry in sorption and ion-exchange processes*. Belgorod. 2010; 103-107.
- [11] Nozdrin GA et al. Physiological status and productivity of geese in the application of probiotics: monograph. Novosibirsk Agrarian University, Novosibirsk, 2017; 194.
- [12] Khmyrov A, Fatyanov A, Gorshkov G. Echinacea and lactobifadol for chicken growth. *Livestock of Russia*. 2012; 9:16-17.
- [13] Yakovleva EG, Gorshkov GI. *Cynoglossum officinale*: monograph. Belgorod, 2009; 135.
- [14] Yakovleva IN, Gorshkov GI, Kushch NN. Histostructure of chicken's liver after addition of aerosil sorbent to their ration. *Agricultural biology*. 2011; 6: 97-102.