

Pharmaceutical Communication

Extracts of Medicinal Plants for Preventing Postnatal Complications in Cows

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ABSTRACT

It is obligatory for every farm to carry out a set of measures in order to detect obstetric and gynecological diseases in the breeding stock of cattle at an early stage, as they can be detected in more than 50% after pathological childbirth. We reported here on determining the effect on the extracts of such medicinal plants as Maral root and stinging nettle, when feeding recipient cows in a dry period, on the prevention of postpartum complications in animals and a reduction in the recovery period of the reproductive system. Dry extracts of the selected medicinal plants containing an increased concentration of the main active substances of the initial plant raw materials were obtained by water-ethanol and liquid extraction with subsequent vacuum drying processing. The effect of extracts on the natural resistance of cows was determined by generally accepted, standard and original research methods using modern laboratory equipment. It was found that on the 30th day in animals that received additional medicinal extracts in the diet, an increase in the content of neutrophils and platelets in the blood and protein fractions of blood serum was observed, which indicates an increase in the resistance of the animal's body. An increase in the bactericidal, lysozyme activity of the blood serum of recipient cows and the phagocytic activity of leukocytes also indicates an increase in the immune response of the animal's body in response to the penetration of foreign microflora into the body. According to the results of the studies, it was recommended to add a two-component Phyto biotic feed additive to the main diet based on extracts of medicinal plants Maral root and stinging nettle at a dose of 50 g per head per day in the ratio of components: 100 kg of compound feed, 0.5 kg of maral root extract, 2, 5 kg of stinging nettle extract.

KEY WORDS: AGE, DYSLIPIDEMIA, GENDER, LIPID PROFILE, SEDENTARY LIFESTYLE.

INTRODUCTION

The development of pathological processes in cows during pregnancy and dry periods often has a negative impact on the resulting offspring, which may be born with various physiological abnormalities or be stillborn (Gilbert, 2019). In this regard, antibiotic therapy for dry cows continues to play an important role in the treatment of infectious diseases and the prevention of new animal diseases. As Animal Health Ireland and many large farms (Sok City (Wisconsin), Eko Niva Agro, etc.) note, the use of antibiotics to treat animals is no longer acceptable. This is due to the fact that there is a high likelihood of developing antibiotic resistance in fetus, which may impede its effective treatment in adulthood (Petushok and Malashko, 2018).

In artificial insemination technology, it is very important to obtain healthy embryos for the use in highly productive animal husbandry. To carry out their qualitative analysis and to establish the effectiveness of embryo transplantation, it is significant to get "clean" embryos without residual antibiotics obtained with colostrum. Medicinal plant raw materials, as a full-fledged source of biologically active substances, are a popular component in animal diets (Ezzat et al., 2016) used to increase the body's natural resistance to infectious diseases that are often manifested in cows in the postpartum period, and can serve as a good substitute for antibacterial therapy. The high demand for medicinal plant materials is explained by their wide availability in almost all parts of the planet, low cost, lack of side effects on the animal's body, high pharmacological and functional action (Zubova et al., 2019; Ulrikh et al., 2019; Lombardi et al., 2020).

The natural resistance of the organism reflects a complex of specific and nonspecific factors caused by the interaction of innate and adaptive immune responses (De Pablo-Maiso et

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al., 2018). With the industrial keeping of animals, stresses and immune deficiencies contribute to the development of pathologies of the birth and postpartum periods of varying severity, reducing the animal productivity. The introduction of pharmaceutical substances based on herbal extracts into the composition of animal diets in order to correct the birth process and to prevent postpartum complications in animals' body by improving the properties of nutrition is a promising direction for replacing antibiotics and increasing the ecological value of animal products.

Conducting our research, the choice was made in favor of Stinging Nettle, since the leaves of stinging nettle have a rich multivitamin composition. They contain Ascorbic acid (up to 269 mg%), vitamin K (42-45 µg/g), vitamin B2, Pantothenic acid, carotenoids (beta-carotene, xanthophyll, violaxanthin) - up to 50 mg%, urticin glycoside, tanning and protein substances, such organic acids as Formic, Coffeic, P-coumaric and Ferulic ones, nitrogenous substances, amino acids, including essential ones. Aerial part of nettle contains important essential trace elements (Zn, Cu, Mn, Fe) and lead (Pb), as well (El-Haouari and Rosado, 2019). Preparations of stinging nettle stimulate blood clotting increase the percentage of hemoglobin and the number of erythrocytes and have a pronounced tonic effect on the smooth muscles of the uterus and produce a vasoconstrictor effect (Belmaghraoui et al., 2018; Chernyavskikh, 2019; Smulski et al., 2020; Yu et al., 2021).

The validity of the Maral Root use is associated with the phyto-ecdysteroids (420 mg/kg) contained in it - biologically active substances. Ecdysteroids are the most widespread and numerous families of steroid compounds. Phyto-ecdysteroids have a prophylactic effect in the treatment of inflammatory and immunological processes. Ecdysterone and the direct analogs of ecdysteroids are the main active substances with an anabolic effect and are promising for use in agriculture (Głazowska et al. 2018). Rhizomes and roots contain alkaloids, ascorbic acid (0.1%), carotene, tannins (about 5%), the amount of phytoecdysones, essential oil (0.9%), phenolics (11.4%), gums, ascorbic acid (68,8 mg%), inulin, coumarins, organic acids. The above-ground mass contains ascorbic acid, protein, sugars, and organic acids (Alamgir, 2017; Yu et al., 2021).

The unique biological activity of the plant is determined by the combination of a complex of substances: mono- and polysaccharides, inulin, organic acids, phyto-ecdysones, triterpenic saponins (raponticosides), vitamins, phenol carboxylic acids and their derivatives, lignin, tannins, essential oil, alkaloids, flavonoids, anthocyanins, and gums, crystals of calcium oxalate, phosphoric acid salts, macro- and microelements (Skala et al., 2016; Głazowska et al. 2018). The effective biological activity of pharmaceutical substances based on extracts of medicinal plants obtained in the form of a powder is 50-100 times higher than the activity of other drugs or preparations. High activity is obtained due to a complex combination of the main active ingredients (Pat. RU 2739625; Abdel-Lattif et al., 2021).

The aim of the research was to study on the effect of extracts of such medicinal plants as Safflower leuzea and Stinging

Nettle in feeding recipient cows during the dry period for the prevention of postpartum complications in animals. Research objectives are: to determine the extracts' effect on the hematological parameters of the blood of recipient cows, the content of total protein and its fractions in the blood serum of animals, indicators of natural resistance of the blood serum of recipient cows; as well as their impact on the postpartum period indicators of recipient cows and the viability of the embryos obtained; to determine the possible concentration of the introduction of extracts of medicinal plants in the main diet of cows.

MATERIAL AND METHODS

In this study the reproductive system of recipient cows was carried out in the conditions of LLC "Farm Enterprise Mikhailovskoye" (Prokopyevskiy District, Kemerovo Region), on selected healthy Black-and-White recipient cows. Cows were selected taking into account their age, live weight, clinical and physiological condition. For the study, three groups of cows were formed; there were 6 heads in each of them (control, first experimental, second experimental, third experimental). The control group consisted of animals kept in farm conditions and received a traditional diet: flaked wheat, sunflower cake, feed yeast, table salt, monocalcium phosphate, hayage silage of vetch, raw potatoes, meadow or pasture hay in the ratios established in the farm.

Experimental group I - animals were fed with a feed additive in the following ratio of components: 100 kg of compound feed, 0.5 kg of Maral Root extract, 1.0 kg of Stinging Nettle extract. Experimental group II - animals were fed with a feed additive in the following ratio of components: 100 kg of compound feed, 0.5 kg of Maral Root extract, 2.5 kg of Stinging Nettle extract. Experimental group III - animals were fed with a feed additive in the following ratio of the components: 100 kg of compound feed, 0.5 kg of Maral Root extract, 4.0 kg of Stinging Nettle extract. The selected recipient cows were fed with an introduction of the enriched feed additive with the first day of the dry period, the amount of the feed additive was 50 g per head per day. The content of the Maral Root extract in the experimental groups remained unchanged due to the high content of phyto-ecdysteroids in its composition.

The effectiveness of enrichment of the recipient cows' main ration with extracts of Maral Root and Stinging Nettle was determined by indicators of hematological and biochemical composition of blood samples taken from the jugular vein of cows in the morning, in the research laboratory "Biochemical, molecular genetic studies and breeding of farm animals" on the basis of the Federal State Budgetary Educational Institution of Higher Education "Kuzbass State Agricultural Academy". The blood samples were taken from recipient cows before the start of the experiment and after 30, 60 and 90 days of feeding them with feed additives of the Maral Root and Stinging Nettle extracts to the main diet. Hematological blood parameters were determined by using a hematological analyzer VetScan HM5 (ABAXIS, USA). When performing the research, the following research objects were observed:

- Maral Root in accordance with GOST 24027.2-80;
- Stinging Nettle in accordance with GOST 24027.2-80;
- Blood serum of recipient cows, samples were taken in accordance with GOST 34105-2017.

When performing the research there were used the generally accepted standard and original research methods. Hematological blood parameters were determined by using a hematological analyzer VetScan HM5 (ABAXIS, USA). The content of total protein, albumin and globulin fractions was determined by using a biochemical analyzer AU 480 (Beckman Coulter, USA). The natural (nonspecific) resistance of the blood serum of donor cows was determined by the bactericidal indicators according to the nephelometric method proposed by Smirnova and Kuzmin. The lysozyme activity of the blood serum of donor cows was determined

by using an indicator in the form of a dry lyophilized form of *Micrococcus lisodecticus* according to the method of Dorofeychuk. The phagocytic activity of the blood serum of donor cows was determined by the method of Kost and Stenko. The viability of embryos obtained from recipient cows was determined according to GOST 28424-2014. The presence of mastitis in recipient cows was determined according to the "Manual on the diagnosis, therapy and prevention of mastitis in cows" No. 13-5-2/1948. The presence of postpartum subinvolution of the uterus and postpartum endometritis was determined rectally.

RESULTS AND DISCUSSION

Hematological blood parameters of the recipient cows are presented in Table 1 and Table 2.

Table 1. Hematological blood parameters of recipient cows. Part 1.

Indicator	Blood test	Group			
		Control	Experimental group I	Experimental group II	Experimental group III
Leukocytes, (WBC), 10⁹/l	before experiment	9,6±0,48	10,0±0,50	9,8±0,49	9,6±0,48
	30 days	10,8±0,54	10,0±0,50	8,5±0,42	8,6±0,42
	60 days	12,0±0,60	9,8±0,49	7,0±0,35	6,8±0,34
	90 days	12,6±0,63	9,5±0,47	6,8±0,34	6,8±0,34
Leukocyte formula, % Basophils Eosinophils Neutrophils Lymphocytes Monocytes	before experiment	-	-	-	-
		3,0	4,0	3,0	3,0
		39,0	38,0	39,0	40,0
		54,0	53,0	54,0	54,0
	30 days	5,0	4,0	4,0	5,0
		-	-	-	-
		3,0	4,0	4,0	3,0
		39,0	39,0	43,0	44,0
	60 days	54,0	52,0	50,0	50,0
		5,0	5,0	5,0	6,0
		-	-	-	-
		3,0	4,0	4,0	3,0
	90 days	38,0	41,0	43,0	45,0
		54,0	52,0	49,0	49,0
		5,0	5,0	5,0	5,0
		-	-	-	-
30 days	3,0	3,0	4,0	3,0	
	38,0	42,0	44,0	45,0	
	54,0	50,0	49,0	49,0	
	6,0	6,0	7,0	6,0	

Changes in hematological blood parameters are associated with an increase in the immunobiological status of animals. As can be seen from Table 1, the additional introduction of Maral Root and Stinging Nettle extracts into the diet of recipient cows has a significant effect on the change in hematological blood parameters. Thus, the quantitative indicators of leukocytes in the control group increased from $9,6 \cdot 10^9/l$ to $12,6 \cdot 10^9/l$ (44%), while in experimental groups I, II and III this indicator decreased by 5%, 31% and 30%, respectively. The content of lymphocytes in the control group remained unchanged throughout the experiment; in the experimental group I the number of leukocytes decreased by 6%, in the experimental groups II and III - by 10%. Since neutrophils have an ability to recognize any bacteria that enter the body, it is important to increase this indicator in the blood to help the body

fighting with extraneous microflora. In the control group, at the end of the experiment, there was no increase in the content of neutrophils; while in the experimental group II, the number of neutrophils in the blood of recipient cows increased by 13%.

The normal development of the functional capabilities of the animal's body largely depends on the optimal functional activity of platelets, which determines a high level of organism's resistance to infectious diseases and a sufficient rate of metabolic processes. The introduction of extracts of Maral Root and Stinging Nettle into the diet of recipient cows allows increasing the platelet content of in the blood by 8%, 15% and 17% in the experimental groups I, II and III, respectively. The erythrocytes and hemoglobin parameters throughout the experiment period

were within the physiological norm in all the groups both at the beginning and at the end of the study. The blood serum was tested to determine the content of total protein, albumin

and globulin fractions by using a biochemical analyzer AU 480 (Beckman Coulter, USA). The biochemical parameters of the blood of recipient cows are presented in Table 3.

Table 2. Hematological blood parameters of recipient cows. Part 2.

Indicator	Blood test	Group			
		Control	Experimental group I	Experimental group II	Experimental group III
Erythrocytes, (RBC), $10^{12}/l$	before experiment	6,9±0,34	6,6±0,33	6,2±0,31	6,3±0,32
	30 days	6,6±0,33	6,4±0,32	5,9±0,29	6,8±0,34
	60 days	6,6±0,33	6,0±0,30	6,5±0,32	6,0±0,30
	90 days	6,2±0,33	6,4±0,32	6,8±0,34	6,8±0,34
Hemoglobin, (HGB), g/100ml	before experiment	11,0±0,55	11,2±0,56	10,7±0,53	10,5±0,54
	30 days	11,2±0,56	11,2±0,56	10,3±0,53	10,9±0,54
	60 days	11,6±0,60	11,2±0,56	9,9±0,49	11,3±0,56
	90 days	11,7±0,60	11,4±0,57	10,3±0,53	10,2±0,53
Platelets, $\times 10^9/l$	before experiment	456±22,8	468±23,4	462±23,1	452±22,6
	30 days	450±22,5	488±24,4	517±25,8	486±24,3
	60 days	443±22,1	500±25,0	530±26,5	522±26,1
	90 days	448±22,4	506±25,3	533±26,6	530±26,5
Hematocrit, %	before experiment	40,50±2,03	40,48±2,03	41,43±2,07	40,52±2,03
	30 days	41,12±2,06	40,23±2,01	41,06±2,05	40,46±2,02
	60 days	41,56±2,07	40,44±2,02	40,72±2,04	40,46±2,02
	90 days	41,88±2,10	40,12±2,00	40,15±2,01	41,12±2,00
Cellular hemoglobin content, (picograms) pg	before experiment	14,4±0,72	14,2±0,72	14,0±0,70	13,8±0,70
	30 days	13,6±0,68	13,2±0,66	14,0±0,70	14,2±0,72
	60 days	14,0±0,70	13,4±0,67	13,8±0,70	13,7±0,67
	90 days	13,9±0,70	13,2±0,66	14,2±0,72	13,8±0,68
Corpuscular hemoglobin concentration, g/l	before experiment	56,4±2,82	56,1±2,80	56,2±2,81	55,7±2,78
	30 days	56,0±2,80	55,6±2,83	55,8±2,79	55,2±2,76
	60 days	56,6±2,83	55,6±2,83	55,2±2,76	56,2±2,81
	90 days	55,7±2,78	55,6±2,83	56,0±2,80	55,9±2,79

Table 2 presents data on studies of blood serum protein fractions. All the noted indicators were within the normal range. Blood proteins have a direct effect on the metabolism in the body of animals. The content of total protein in the blood and its fractions allows making a conclusion about the physiological state of recipient cows, as well as the body's resistance to unfavorable environmental factors. Extracts of Maral Root and Stinging Nettle, when added to the cow main diet, increase the total protein content in the blood by 10% compared to the control group, where this indicator practically does not change. Many scientists point out the connection between the content of albumin in the blood of animals with their productivity (Lacasse et al., 2018; Lombardi et al., 2020; Fallah et al., 2021).

The use of extracts of medicinal plants in the feeding of recipient cows makes it possible to increase the content of the albumin fraction in the blood by 2-3%. The carotene content in the blood of animals from the experimental groups increased almost 2 times compared with the control group and amounted to 0.50-0.52 mg%. A low level of carotene in the blood serum of cows is the cause of hypocarotemia - insufficient supply of provitamin A in the diet, when there is a lack of protein and easily digestible

carbohydrates, and B vitamins in the feed. Lack of carotene in the blood of recipient cows can lead to reproductive function abnormality of the animal, poor heat and prolonged ovulation (Petushok and Malashko, 2018; Smulski et al. 2020; Abdel-Lattif et al., 2021). During the experiment, the effect of Maral Root and Stinging Nettle extracts on the phagocytic activity of leukocytes, lysozyme and bactericidal activity of blood serum of recipient cows, which have a direct effect on the animal body natural resistance, was determined, as well (Table 4).

The bactericidal activity of blood serum is one of the important indicators of the body's natural resistance to viral diseases. This parameter shows the ability to suppress the growth of microorganisms and depends on the activity of all humoral resistance factors (Kirikovich et al., 2012; Tresnitsky, 2019). The bactericidal activity of blood serum in the recipient cows of the control group did not change during the experiment; in all experimental groups, it increased by 40%, 94% and 87%, respectively. The lysozyme activity of blood serum also, in turn, makes it possible to characterize the natural resistance of the organism (Islam et al., 2017; Eremenko and Rotmistrovskaya, 2021).

Table 3. The content of total protein and its fractions in blood serum

Indicator	Blood test	Group Control	Experimental group I	Experimental group II	Experimental group III
Total protein, г/л	before experiment	73,89±3,69	73,27±3,66	73,62±3,68	73,55±3,68
	30 days	74,15±3,70	74,33±3,72	76,16±3,81	78,71±3,93
	60 days	74,32±3,72	74,90±3,74	81,00±4,05	80,79±4,04
	90 days	74,43±3,72	75,00±3,75	80,98±4,05	81,24±4,06
Carotene, mg%	before experiment	0,25±0,01	0,26±0,01	0,25±0,01	0,25±0,01
	30 days	0,25±0,01	0,28±0,02	0,38±0,03	0,42±0,02
	60 days	0,28±0,02	0,28±0,02	0,50±0,03	0,49±0,03
	90 days	0,29±0,02	0,31±0,02	0,52±0,03	0,50±0,03
Albumin, %	before experiment	37,12±1,86	37,15±1,86	37,22±1,86	37,12±1,86
	30 days	37,00±1,86	37,34±1,87	38,00±1,90	37,88±1,89
	60 days	36,88±1,85	37,88±1,89	38,12±1,91	38,14±1,91
	90 days	36,92±1,85	37,87±1,89	38,10±1,91	38,14±1,91
α-globulins, %	before experiment	12,46±0,62	12,42±0,62	12,35±0,61	12,40±0,62
	30 days	12,46±0,62	12,46±0,62	12,40±0,62	12,42±0,62
	60 days	12,53±0,63	12,58±0,62	12,48±0,63	12,42±0,62
	90 days	12,50±0,62	12,56±0,62	12,50±0,63	12,50±0,63
β-globulins, %	before experiment	13,87±0,69	14,15±0,71	14,33±0,72	14,60±0,73
	30 days	13,92±0,70	13,96±0,70	14,18±0,71	14,42±0,72
	60 days	13,84±0,69	14,00±0,70	14,18±0,71	13,96±0,70
	90 days	13,85±0,69	14,10±0,71	14,25±0,72	14,15±0,71
γ-globulins, %	before experiment	36,04±1,80	35,57±1,78	35,77±1,79	36,66±1,83
	30 days	36,16±1,81	35,85±1,79	35,26±1,76	36,21±1,81
	60 days	35,77±1,79	36,26±1,81	34,58±1,73	35,96±1,80
	90 days	35,59±1,78	36,14±1,81	34,32±1,72	35,96±1,80

Table 4. Indicators of natural resistance of blood serum of recipient cows

Indicator	Blood test	Group Control	Experimental group I	Experimental group II	Experimental group III
Serum bactericidal activity, %	before experiment	29,30±1,46	28,68±1,43	27,83±1,40	29,62±1,48
	30 days	28,53±1,43	34,16±1,71	38,33±1,92	39,42±1,97
	60 days	29,22±1,46	38,49±1,92	50,17±2,51	51,39±2,57
	90 days	29,61±1,47	40,06±2,00	53,93±2,70	55,38±2,77
Serum lysozyme activity, %	before experiment	13,82±0,69	13,68±0,69	13,90±0,69	14,01±0,70
	30 days	13,75±0,69	13,70±0,69	14,47±0,72	14,34±0,72
	60 days	13,00±0,65	13,96±0,70	15,00±0,74	14,73±0,74
	90 days	13,00±0,65	13,74±0,69	14,89±0,74	14,80±0,74
Phagocytic activity of leukocytes, %	before experiment	63,58±3,18	63,29±3,16	63,65±3,18	62,79±3,14
	30 days	63,38±3,17	63,57±3,17	64,65±3,23	63,80±3,19
	60 days	64,00±3,20	64,36±3,16	65,60±3,28	64,00±3,20
	90 days	63,72±3,19	64,36±3,22	65,72±3,29	64,00±3,20

Table 5. Indicators of the postpartum period of recipient cows

Indicator	Control group	Experimental group II
Duration of the placental stage, hour	8,21±0,41	5,30±0,26
Postpartum subinvolution of the uterus, heads	2	-
Postpartum endometritis, heads	1	-
Serous mastitis, heads	1	-
Number of animals for subsequent embryo transfer, heads	6	10

Table 6. Determination of the viability of the obtained embryos

Indicator	Control group	Experimental group II
Number of born calves, incl.	6	6
healthy	4	6
stillborn	1	-
with physiological abnormalities	1	-

Lysozyme activity in all experimental groups increased on average by 5-7%, while this indicator in the control group decreased by 6%. The addition of Maral Root and Stinging Nettle extracts to the main feeding ration of recipient cows also contributes to an increase in the phagocytic activity of leukocytes in the blood by 2-4% compared to the control group. The increase in the intensity of the phagocytic activity should be associated with an increase in the immune response from the body, in response to the penetration of foreign microflora inside the organism (Ulrikh et al., 2020; Eremenko and Rotmistrovskaya, 2021).

After analyzing the obtained data of morphological and biochemical parameters of blood got from recipient cows feeding with different concentrations of Maral Root and Stinging Nettle extracts, it was established that the optimal feed additive should be prepared in the following ratio of components: 100 kg of compound feed, 0.5 kg of extract of Maral Root, 2.5 kg of Stinging Nettle extract. An increase in the concentration of the extract of Stinging Nettle in the feed additive does not have a significant effect on the hematological parameters of blood and indicators of natural resistance of blood serum. At the same time, a decrease in the dose of the extract does not allow achieving the desired result; the morphological and biochemical parameters of the blood practically do not differ from the control group. In addition to studying the morphological and biochemical parameters of blood, recipient cows of the control and experimental group II were analyzed on the point of the duration placental stage, the timing of uterus involution, the period from calving to the embryo introduction (Table 5).

As can be seen from Table 4, in the experimental group II, the duration of the placental stage was reduced by 36%. In addition, in the control group, which received only the basic diet, postpartum complications were noted in 4 cows (40%). Out of 10 cows-recipients of the control group, 2

animals after calving were diagnosed with subinvolution of the uterus (20%), 1 cow (10%) had postpartum endometritis, and one animal had serous mastitis (10%). In animals of the experimental group, no signs of postpartum complications were revealed, this fact confirms the effectiveness of the introduction of the selected extracts of medicinal plants into the diet of recipient cows. In order to confirm the effectiveness of herbal extracts as a substitute for antibiotics in feeding dry cows and, as a consequence, to obtain healthy young animals, embryos obtained from recipient cows of the control and experimental group II were analyzed (Table 6).

According to the presented results, it was found that the introduction of extracts of medicinal plants into the main diet of recipient cows makes it possible to obtain healthy embryos without pathological changes. At the same time, 16% of embryos obtained from recipient cows of the control group had physiological abnormalities, in particular, they were hypotrophic, and 16% of embryos were stillborn.

CONCLUSION

After analyzing the obtained data of morphological and biochemical parameters of blood, depending on the concentration of the Maral Root and Stinging Nettle extracts in the feed additive for recipient cows, it was found that the feed additive is introduced to the animals in the ratio of the components: 100 kg of compound feed, 0.5 kg of Maral Root extract, 2, 5 kg of Stinging Nettle extract, which enhances the immune response from the animal's body, in response to the penetration of foreign microflora inside the organism.

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