RESEARCH RESULT
НАУЧНЫЙ РЕЗУЛЬТАТ
Yakovleva I.N., Shaposhnikov A.A., Vesenzev A.I., Kovaleva V.U., Zakirova L.R., Shevchenko T.S., Shentseva E.A. A Method for production of phytomineralsorbent, physical and chemical properties of it, effect on the living systems and the quality of the livestock industry products Research result: pharmacology and clinical pharmacology. 2016. Vol. 2, №2: 119-124.

Рус.

UDC: 636.087.7 **DOI: 10.18413/2313-8971-2016-2-2-119-124**

Yakovleva I.N.[1]
Shaposhnikov A.A.[2]
Vesenzev A.I.[3]
Kovaleva V. U.[4]
Zakirova L. R.[5]
Shevchenko T.S.[6]
Shentseva E.A.[7]

A METHOD FOR PRODUCTION OF PHYTOMINERALSORBENT, PHYSICAL AND CHEMICAL PROPERTIES OF IT, EFFECT ON THE LIVING SYSTEMS AND THE QUALITY OF THE LIVESTOCK INDUSTRY PRODUCTS

1) PhD of Biological Sciences, associate professor , the chair of the Department of the non-infectious pathology of V.Y. Gorin Belgorod State Agrarian University. 1, Vavilova St., Mayskiy Settl., Belgorod Reg., 308503, Russia. e-mail: inka707@mail.ru
2) Doctor of Biological Sciences, professor, chair of Biochemistry department of Belgorod State National Research University 85, Pobedy St., Belgorod, 308015, Russia. e-mail: shaposhnikov@bsu.edu.ru
3) Doctor of Technology Sciences, professor, chair of department of General Chemistry of Belgorod State National Research University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: vesentsev@bsu.edu.ru
4) PhD of Biological Sciences, associate professor of the Morphology and Physiology Department of V.Y. Gorin Belgorod State Agrarian University. 1, Vavilova St., Mayskiy Settl., Belgorod Reg., 308503, Russia
5) PhD in Biological Sciences, associate professor of the Biochemistry Department of Belgorod State National Research University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: zakirova@bsu.edu.ru
6) PhD in Biological Sciences, senior lecturer of the Biochemistry Department of Belgorod State National Research University 85, Pobedy St., Belgorod, 308015, Russia. e-mail: shevchenko_ts@bsu.edu.ru
7) PhD in Biological Sciences, associate professor of the Biochemistry Department of Belgorod State National Research University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: shentseva@bsu.edu.ru

Abstract. Numerous experimental studies have shown that the sorption-active substances can clarify the reservoir, soils, drinking municipal water from unwanted impurities with a sufficient degree of effectiveness; eliminate the potentially toxic for health chemicals of different chemical nature from human body. This is ensuring their using in the animal production practice, veterinary medicine and medicine. The main purpose and focus of this study are associated with the solution of problems of the maintaining physiological and biochemical status of live-stock animals. It provides their productivity and biological value of obtained products.

Key words: montmorillonite clay, medicinal plants, metal ascorbates, enterocorbents, opportunistic pathogenic microflora, piglets, blood biochemistry, the quality of the livestock industry products.

Introduction.

The previously conducted studies proved the perspective of combined use of mineral sorbents and cellular juices for maintain the immune status of the animals, the enhancement of the metabolic pathways in the organism. [1, 2, 3, 4] We have decided to supplement the information available to us about the effect of phytomineralosorbent (PMS) on living systems in the present study. We chose two directions of using new composites: 1) administration as enterosorbents; 2) creation of new medicinal substances for the local and system treatment of purulent wounds different ethiology.

Materials and methods.

In present study the sample of montmorillonite clay from Polyana deposits in Shebekincky district of Belgorod region. Research clay had light greenish tinct and it was the solid conchoidal fracture rock. Chemical composition of clay was stydied by methods of X-ray fluorescent analysis (X-ray spectrometer ARL OPTIM'X) and x-ray spectral microanalysis (analyzer EDAX, combined with the scanning electron microscope Quanta 200 3D). An analysis of the mineralogical composition of the samples was performed on the X-ray diffractometer Rigaku Ultima XRD 320, and by using transmission electron microscope JEM 2100. Specific surface of material was determined by the method of nitrogen

low-temperature adsorption (analazer of specific surface area - TriStar).

Other objects of the research were medicinal plants: common Saint-John's wort (Hypericum perforatum) and bloodwort (Achillea millefolium) that have styptic and antimicrobial properties; the flowers of pot marigold (Calendula officinalis) with antispastic and antiseptic active compounds in the composition; purple coneflower (Echinacea purpurea), which contained the substance of polysaccharide nature with the immunomodulating properties.

Herbal raw materials harvested by techniques adopted in pharmacognosy: the tops of flowering yarrow and St. John's wort (25-30 cm long without serious lower portions of the stems) were cut with scissors; grass of cultivated Echinacea purpurea was mowed; the flowers of pot baskets marigold were terminated manually. Collection dates were determined by the onset of vegetative phases, when the largest number of active substances contained in the studied plants [5]. Processing of raw materials led in the day after the harvesting of sampling for chemical analysis to extract the juice. At the same time the phasing and the basic technological methods of wet fractionation plant, developed in relation to forage plants were observed [6].

Activated montmorillonite and cellular juice, mixed in a ratio of 1.3: 1 to 1.5:1 were used to obtain qualitative PMS (figure 1).

We mixed the powdered microbeads of PMS with solution of ascorbate complex of iron, zinc, manganese and cobalt to produce phytoascomineralosorben (PAMS).

After drying paste mass and turning of it to solid state of the aggregative condition produced PAMS was pebbled.

Chelates are the most favorable form of metal assimilation in the body. These solutions are chelated metal complexes were in the composition of PAMS, they are developed by scientists of the Belgorod State Agricultural Academy [7, 8, 9].

In the next series of experiments we studied the PAMS sorption. For that we prepared different model solutions, the concentration 0,1 M\l of standard solutions of transition metal nitrates (Pb, Cu, Cd) was got by solubilizing of pure metal (not less than 99,9 % of the main compound) in concentrated nitric acid

The solutions were evaporated to complete removal of HNO_3 in a calibrated flask and double diluted with distilled water to the required volume.

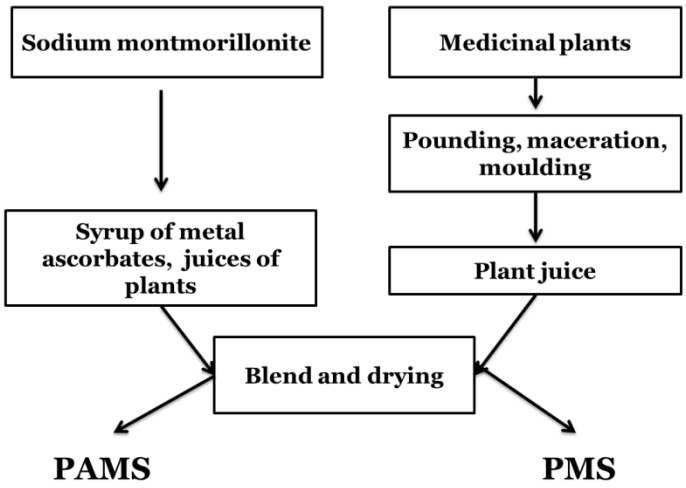

Figure 1. The scheme for PMS and PAMS production.

Model solutions were the aqueous solutions that contained: a). HNO_3 and $NaNO_3$; b). HCl, NaCl and H_2NCH_2COOH; c). HCl $NaHCO_3$. Selection of the composition model solutions was due to the presence of components in the gastrointestinal tract (Na^+, Cl^-, NO_3^-, HCO_3^-, CO_3^-amino acids). Salt content in model solutions was supported by constant and the equal 1 % (by mass). With a view to variability of pH content of acids was changed from 0 to $2*^{10-2}$ M\l.

The solution of the compounds that were necessary for of the model solutions, we prepared from concentrated solutions of HCl and HNO_3 or from associated solid compounds according to the qualification classification “chemically-pure”.

In determining the antimicrobial activity of the juices from freshly and aqueous extracts from dried raw herbs, and suspension phytomineralosorbt supplements. Daily culture gram-negative, gram-

positive and spore-forming microflora were used as test-microorganisms. Liquids studied for antimicrobial activity, impregnated with sterile filter paper disc and then we put it on meat-and-peptone agar (in the Petri dishes), planted with test-microorganisms. The results were assessed after the daily cultivation at a temperature of 37º Celsius degree.

The arrest of development zone size (evolving the disc (8 mm) diameter and the field of culture where there were no signs of growth around it) was considered as a qualitative indicator of extent of phytoagent antimicrobial activity.

Absence of such zones is interpreted by us as an inability of separated phyto-agent exerts bacteriostatic action on a certain kind of test- culture.

The chemical composition of plant raw materials, fractionation products and PMS and PAMS was determined by conventional feed analysis and feed supplements methods.

The results of the study and their discussion.

It was found that the chemical content of the clay is represented the next oxides, mass%: SiO_2 – 62,60; Al_2O_3 – 18,62; Fe_2O_3 – 5,81; MgO – 3,22; CaO – 5,53; Na_2O – 0,40; K_2O – 2,91; TiO_2 – 0,91. Enough high content of calcium and iron oxides, can indicate the presence of calcium glandular form of montmorillonite in the mineralogical composition of the clay.

The high calcium oxides content determines calcite mineral, which presence in the sample.

Such natural raw materials can be considered as polymineral clays because their phase composition is represented by the following components: montmorillonite (15.566; 10.231; 6.813; 2.160; 2.127; 1.991; 1.773; 1.671; 1.501 Å), crystalline silica (4.256; 3.343; 2.456; 2.281; 2.237; 2.127; 1.979; 1.817; 1.441 Å), illite (9.923; 5.103; 4,171, 3.243 Å), kaolinite (7.899; 4.171, 2,254; 1.625 Å), mirror stone (4.968; 3.954; 1.658; 1.578 Å), calci-spar (3.036; 2.842;1.604;1.587 Å), clinoptilolite (8.927; 3.562; 3.127; 2.513 Å).

A more bright-field electron-microscopic detailed study of the clay suspension deposit is in the Belgorod region, carried out by the combination of microdiffraction and energy-dispersive chemical content determination, and showed that the basic rock-forming mineral in the clay is montmorillonite. The particles of this mineral in the formulations have a vague cloud-like appearance, which is typical for montmorillonite.

The particles of this mineral in the supplements have a blurred cloud-like habit, which is typical for montmorillonite. Their size ranges from fractions to 3-5 microns. All particles of montmorillonite have a polycrystalline structure.

The particles of montmorillonite from Maslopristan' (deposit) clay were present on the energy-dispersive spectrum as peaks: Si, O, Al, Ca and Fe, and the concentration of these elements were 51,8; 33,9; 9,52; 4,3 and 0,4 mass% accordingly. All of this points to calcium variety of the mineral.

According to the findings we came to the following conclusions. The first: sufficient high sorption capacity towards the ions Pb^{2+}, Cu^{2+} and 137 Cs^{+} of the object of the experiment provides an opportunity to use montmorillonite sorbent for decrease concentrations of described toxicants; the use of the sorbent for recovery of Cd2 + is less effective. The second: the results of the sorption kinetics study in two hours castro-intestine intended transit time showed that sorption capabilities towards Pb^{2+}, Cu^{2+}, Cd^{2+} montmorillonite sorbent is realized by 90% or more.

The main factors determining the sorption properties of the original montmorillonite sorbent are: pH, temperature, nature of the sorbed ion and its concentration, the composition of the model solution (in vivo - the composition of the contents of the stomach and intestine) and the sorbent composition.

By its sorption activity towards the model sorbent cations are arranged in sequence: $Pb^{2+} > Cu^{2+} > Cd^{2+}$. The sorption properties of the studied montmorillonite are enhanced by: an increase in pH value, an increase in temperature and decrease in the concentration of sorbed ions; negative – presence components interacting with the research transition metals in solution (including metals and ascorbates herbs juices

Almost complete removal of lead, copper and cadmium is achieved with approximately the following conditions: $pH > 5$, $c(Pb^{2+}) < 80$ mg/l, $c(Cu^{2+}) < 25$ mg/l, $c(Cd^{2+}) < 1,2$ mg/l. Consequently, due to the practical use of a sorbent or supplements on its basis transition metals extraction will occur in different parts of the intestine ($5 < pH < 8$) to ensure that the destruction of metal complexes with donor-active feed components (figure 2).

In addition to data on montmorillonite, it seemed also necessary to determine the chemical composition of the original medicinal plant and its fractionation products.

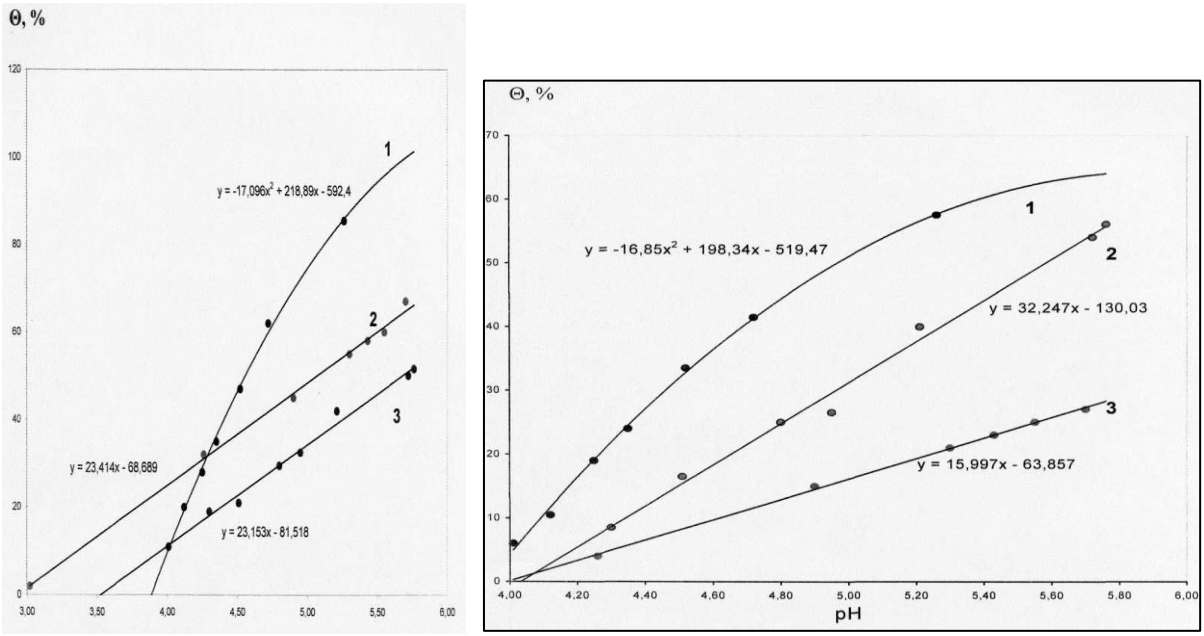

Figure 2. The dependence of the degree of extraction of Pb^{2+} (A) and Cu^{2+} (B) in the systems HNO_3+$NaNO_3$ (1); HCl+NaCl+glycine (2); HCl+$NaHCO_3$ (3).

Table 1 presents the results of phosphorus and other biologically active substances content in the feedstock and products of their wet fractionation.

Concentration of minerals and biologically active substances in the juice is higher than the press residue when calculating the received results for juices on the dry matter.

Table 1

The concentration of phosphorus and biologically active substances in medicinal plants and in the products of their fractionation

Parametrs	Phosphorus	Carotenoids	Flavanoid compounds (calculated as rutin)	Tannins (calculated as tanin)	Polysaccarides
Achillea millefolium					
Intact plant	2,32	19,62	10,17	37,10	–
Press-residue	2,30	16,16	3,35	4,44	–
Juice *	5,19	8,52	6,42	22,27	–
Calendula officinalis					
Intact plant	3,86	19,11	17,50	n.d.	–
Press-residue	3,00	12,30	4,19	n.d.	–
Juice *	0,42	0,25	9,92	n.d.	–
Hypericum perforatum					
Intact plant	3,40	17,98	39,00	68,22	–
Press- residue	2,30	16,31	10,31	10,57	–
Juice *	1,51	1,31	22,47	42,45	–
Echinacea Purpurea					
Intact plant	2,90	10,45	1,33	31,60	36,93
Press- residue	2,73	8,99	0,43	8,41	21,65
Juice*	0,50	1,88	0,53	12,75	9,98

Note: kg^{-1} of mass calculated as a dry substance; * - on natural humidity; a dash - not determined; n.d. - not detected

The chelate nature of iron, zinc, cobalt and manganese ascorbates was confirmed by IR spectral analysis [7, 8, 9]. Their supposed structural formula is shown in the example of cobalt ascorbate in figure 2.

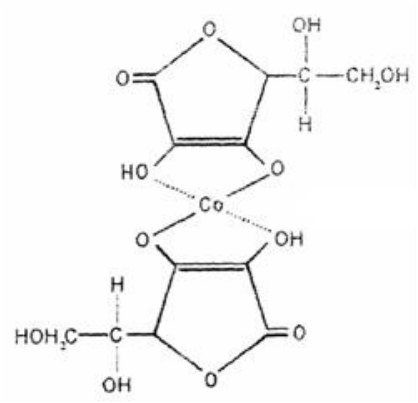

Figure 3. Cobalt ascorbate

In the series of experiments for determine the sensitivity to the juices of herbs of the test cultures (staphylococcus, streptococcus, escherichia, salmonella and bacillus) juice of yarrow was the best. The greatest activity of it was observed in regard to Streptococcus. The reduced antibacterial activity for the studied cultures of microorganisms was observed by the juices of grass St. John's wort and marigold flowers. Only staphylococci showed high sensitivity to the juice of echinace. The remaining bacteria were resistant. Test different combinations of juice mixture did not cause higher activity in comparison with the juices of separated plants.

The bacteriostatic effect of infusions and decoctions of these medicinal plants was higher than the juice.

Suspensions and powders of PMS and PAMS not inhibited proliferation of the test culture, they stimulated their growth surrounding disks.

These PAMS tested on piglets. It was investigated the chemical composition of the diet of piglets and a separated content of PAMS in accordance with the detailed rules adopted by the feeding of live-stock animals in the country. It included: whole protein; crude fat; crude fiber; crude ash; carotenoids; flavonoids; tannins; polysaccharides; and natural and synthetic bioorganic complexes of zinc; manganese; iron, cobalt, ascorbic acid.

The data of piglets' blood test revealed the changes in hematopoiesis, immune and metabolic status of the animals when the different doses of PAMS were added to the feed (g/kg of combination fodder). Thus, the hemoglobin concentration was significantly increased in two months of age pigslets. The level of immunoglobulins in the serum of 68-day-old piglets, in diet of which was combination fodder containing 2 and 3 g PAMS per 1 kg of feed, was reduced by 23 and 13% accordingly. Content of albumin and beta globulins was increased by 4.28 to 3.04%, but the level of gamma globulins was decreased by 7.35% in blood serum of 3-month piglets under influence of PAMS in dose 3g per kg of feed.

The concentrations of vitamins C and E were decreased by 14.3 and 6.4% with a statistically significant increase in the level of vitamin A in the liver by 7.4% ($p \leq 0.05$).

Lysozyme activity of blood serum varied by age periods and significant changes has not been registered in this indicator under the influence of feeding PAMS.

Nutritional intervention of PAMS at doses of 1, 2, and 3 g/kg in diet of piglets first three months of life reduced the incidence of gastroenteritis as compared with control 20, 30 and 35%, accordingly.

Weight gain of piglets under the influence of sorptive bioactive supplement PAMS was higher than in control group of animals.

PAMS use in feed composition at a dose of 3g/kg significantly reduced the cadmium concentration in kidney and bone at 9.8 and 17.3%, accordingly. Also there was a decreasing trend in the content of lead, copper and cadmium in liver, kidney, muscle and blood.

Conclutions.

Native clay deposit Belgorod region is the main raw material multimineral rock-forming mineral - montmorillonite having a particle size fraction of 3-5 mm and a polycrystalline structure .In the in vitro conditions expressed clay has absorption properties that are most manifest in a weakly acidic medium and are expressed at least low pH. This suggests that the sorbent is not active in the stomach (pH 1.8-2.0), and sorption of heavy metal will flow in the small (reduced risk of suction into the blood metals) and large sections of the intestine.

The combination of montmorillonite with cellular juice medicinal plants, chelate metal complexes and ascorbic acid significantly reduced

the sorption quality of the composite and did not contribute to the active inhibition of pathogenic microorganisms.

It presents data on the chemical composition of phyto- and phytoascomineralosorbents and it shows their prospects for the use in livestock industry. This will be confirmed experimentally by us.

Introduction of different doses of PAMS in the diet of piglets between the ages of 14 to 99 days leads to positive changes in the intensity of the growth and development of the animals; maintaining their normal physiological condition, adapted to the conditions of industrial technology.

On the basis of physiological and biochemical studies data it is established that the optimal dose of phytomineralosorbent for piglets aged up to 3 months - 3 g/kg of feed.

Reference

1. Kovaleva V.Ju., Shaposhnikov A.A., Buhanov V.D., Volkov, I.N. The chemical composition of certain types of plant raw materials and derived juices. Problems of agricultural production at the present stage and their solutions (2003): P. 133-134 [eLIBRARY]

2. Shaposhnikov A.A., Klochkova G.N., Zakirova L.R. [et al.]. Study of phytosorbents on biochemical parametres of blood serum of broiler chickens in the preclinical trial. Belgorod State University Scientific Bulletin: Natural sciences. Vol 7(160) (2013): P. 92-96 [eLIBRARY][Full text]

3. Shaposhnikov A.A., Vezentsev A.I., Volovicheva N.V. [et al.]. Physical and chemical characteristics of the sorbent based on montmorillonite-containing clay and its combination with ascorbates of biogenic elements. Bulletin of Voronezh State University. Series: chemistry, biology, pharmacy. Vol. 6 (2012): P. 67-71

4. Shaposhnikov A.A., Buhanov V.D., Zakirova L.R., Yakovleva I.N. [et al.] Structure, properties and effect of the natural enterosorbents on living systems. Sourcebook of all-russian scientific conference with international participation “Sorption and ion-exchange processes in nano- and supramolecular chemistry” Belgorod. (2014): P. 192-194

5. Grinkevich N. I., Ladyginoj E. Ja. Pharmacognosy: Atlas. M.: Medicine. 1989. 512 P.

6. Ponomarev A. F., Alimov T. K., Shaposhnikov A. A. The production and use of fractionating green mass of plants products in livestock. Methodological recommendations. Belgorod. 1990. 40 P

7. Pat. 2078519 Russian Federation, MPK A23K1/16. Feed supplement / Zankevich A.Ju., Bojko I.A., Merzlenko O.V. Shaposhnikov A.A.; declarant and patentee Belgorod Agricultural Institute (educational and scientific center for agriculture). – request 94018287/13 from 18.05.1994, publ. 10.05.1997 [Fips.ru]

8. Pat. 2095995 Russian Federation, MPK A23K1/16 Method of feeding livestock and poultry / Merzlenko O.V., Zankevich A.Ju., Bojko I.A., Kozubova L.A. [et al.]; declarant and patentee Belgorod State Agricultural Academy. – request 96101427/13 from 22.01.1996, publ. 20.11.1997 [Fips.ru]

9. Pat. 2099965 Russian Federation, MPK A23K1/16 Method of feeding livestock and poultry / Ponomarev A.F., Merzlenko O.V., Zankevich A.Ju., Bojko I.A. [et al.]; .]; declarant and patentee Belgorod State Agricultural Academy.– request 96107594/13 from 16.04.1996, publ. 27.12.1997 [Fips.ru]