First International Conference on Humics-based Innovative Technologies «Natural and Synthetic Polyfunctional Compounds and Nanomaterials in Medicine and Biomedical Technologies»

The Mechanisms of Regulatory Influence of Biologically Active Substances of Humic Nature on the Organism of Animals

Stepchenko Liliya
chief of department Physiology and Biochemistry,
Scientific Research Laboratory on behalf of humic substances
prof. L.A. Christeva
Dniepropetrovsk State Agrarian University, Ukraine
stepchenko@rambler.ru

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Ukraine has considerable reserves of peat, which are used primarily in the fuel industry. Composes a valuable peat humic substances are, but their use in Ukraine is practically not carried out. Since 60-th scientists world-famous scientific school of professor L.A. Christeva (Dnepropetrovsk State Agricultural University) were obtained preparations of humic nature and carried out extensive studies on their use in agricultural, animal breeding and poultry. The results of these studies have proven the efficiency of humic substances for the correction of the metabolism, increase productivity and adaptive capacity to process and climatic stresses.

Today extending the application of peat is constrained due to lack of detailed comparisons of physical and chemical properties, chemical composition, toxicity and spectrum of biological activity of peat of different geographical zones of Ukraine.

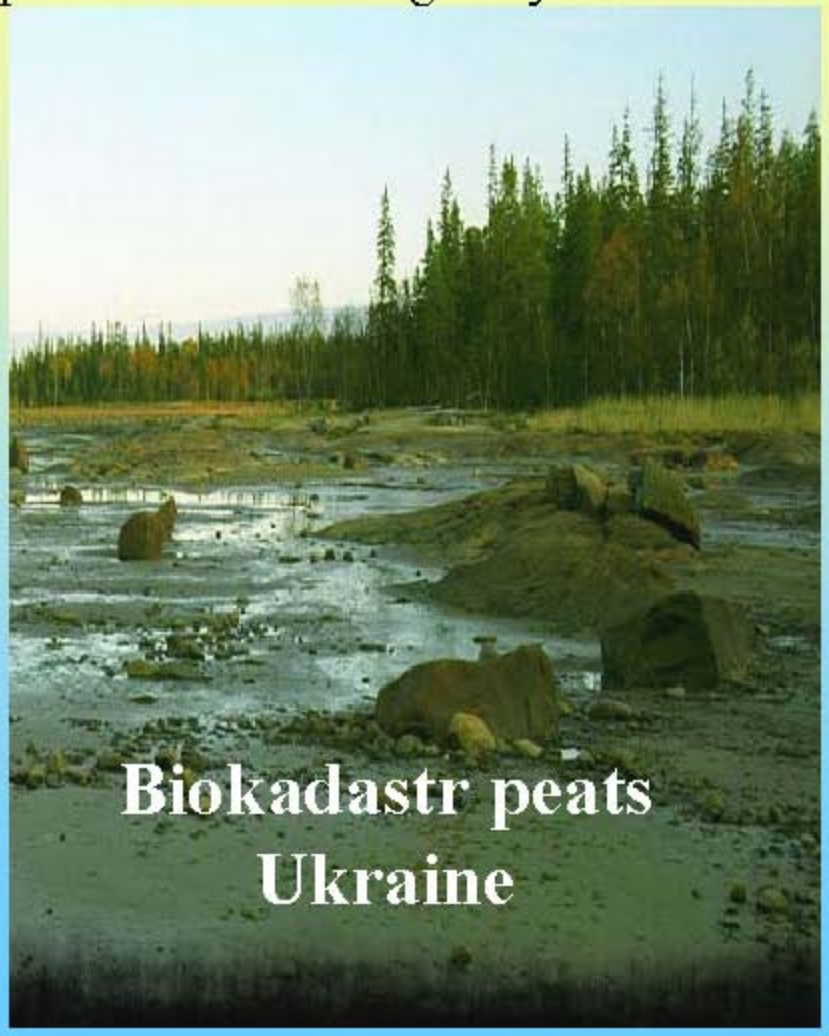
For the purpose of comparison and evaluation of different peat deposits in Ukraine, we developed a screening system

Screening is performed by the following set of tests:

- assessment of physical and chemical properties of raw peat range of indicators;
- estimate the toxicity of raw peat (tests using Paramecium as a test object, parabiotichesky test);
- study of the botanical composition of peat;
- determination of humic acids in the peat;
- activator of growth preparations from peat (influence on biometrics plant vigor seeds, yeast dough, etc.);
- anti-stress activity (tests on heat-, freeze-resistentnost, etc.);
- antioxidant activity, toxic properties;
- enzyme-and immunomodulating activity;
- adaptogenic properties.

Carried out physical-chemical, toxicological, biochemical experimental studies of samples of peat deposits developed different geographical zones of Ukraine.
Defined botanical composition of the peat, set its type, subtype, and type of group.
A set of tests to determine the biological activity of peat.
The study of the biological activity of different botanical composition of peat, peat and various geographical zones of Ukraine.
Based on the results of experimental studies designed structure and concluded Biokadastr peat's from Ukraine on a comprehensive assessment (physical and chemical properties, the level of toxicity and biological activity).

Results of the analysis of peat on screening system formed the basis of peat Biokadastr Ukraine. Biokadastr formed as a systematic information system intended for use as a reference when selecting fields for the production of biologically active substances from peat



Biokadastr can be a science basis for expansion of peat use, rational, science sound management of stocks for biologically active substances, to development modern environmentally sound technologies to obtain new drugs humic nature.

In the Research Laboratory of humic substances in the name of professor L.A. Christeva Dnepropetrovsk State Agrarian University, developed and approved the technical specifications (TS) and approved the recommendations on the application for the next feed of biologically active additives for livestock and poultry: Guminat, hydrohumate, Gumilid, HSVA.

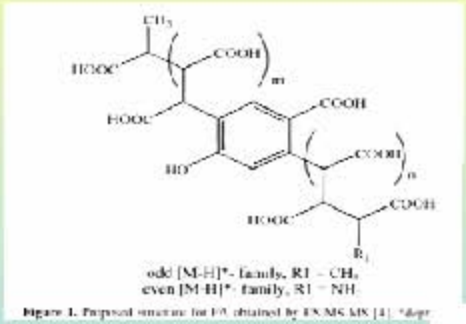
Devepoled us feed additives humic nature not cumulated in animals, show no toxic, embryotoxicity and teratogenicity.

There are several theories about the mechanism of action of humic substances on the organism of animals associated with their separate properties - the effect on the structure of biological membranes and their insights for a variety of substrates, direct participation in the metabolic and bioenergetic reactions, hormone action at the expression of the structural-functional.

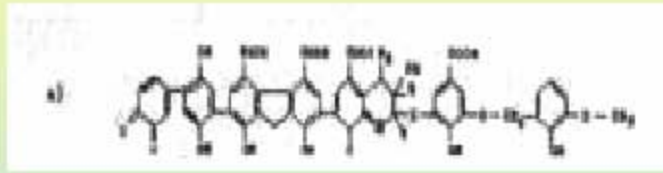
However, the mechanism of action on animal organism is not fully elucidated, so it can be hypothesized on the participation of humic substances in the regulatory processes that ensure the synthesis of biological production in the body of highly productive animals.

In complex molecules of humic compounds are distinguished nuclear and peripheral parts

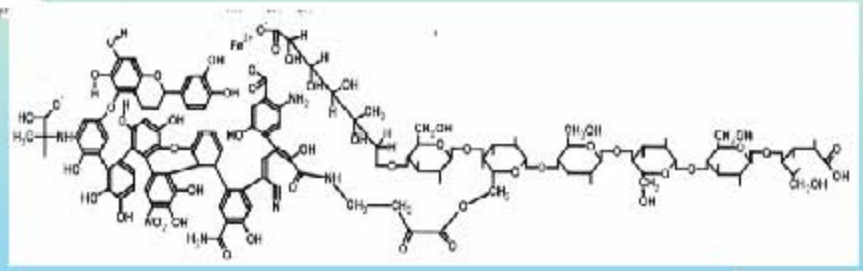
Gipotetichni formulation humic substance



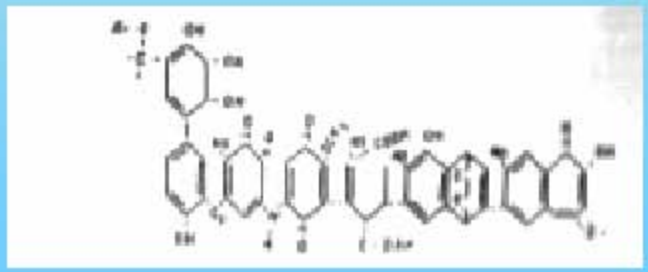
- Moulin (2004)



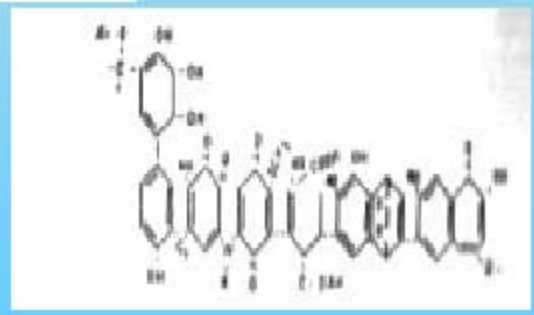
- Rakovskiy (1980)



- Kleinhempel (1992)



- Flyaig (1975)



- Komissarov (1975)

After entering the gastrointestinal tract of the molecules of humic compounds may be subjected to partial biochemical degradation. In the chyme in this case can be appear biologically active functional groups of the peripheral part of the molecule that can pass through the membrane of the intestinal wall. Consequently, after sucking more biologically active fragments can be metabolized in the liver and influence on the processes of regulation of homeostasis of animals.

As for the permeability of the nuclear part of humic compounds, which have a complex polyphenolic nature, it is expressed most opposite opinions. Some researchers Fokine et al, (1985) using tracer have shown that humic compounds are able to pass through the cell membrane. Others (Schnitzer M.), argue that such a process is impossible because of the fairly large size of the molecules of humic compounds.

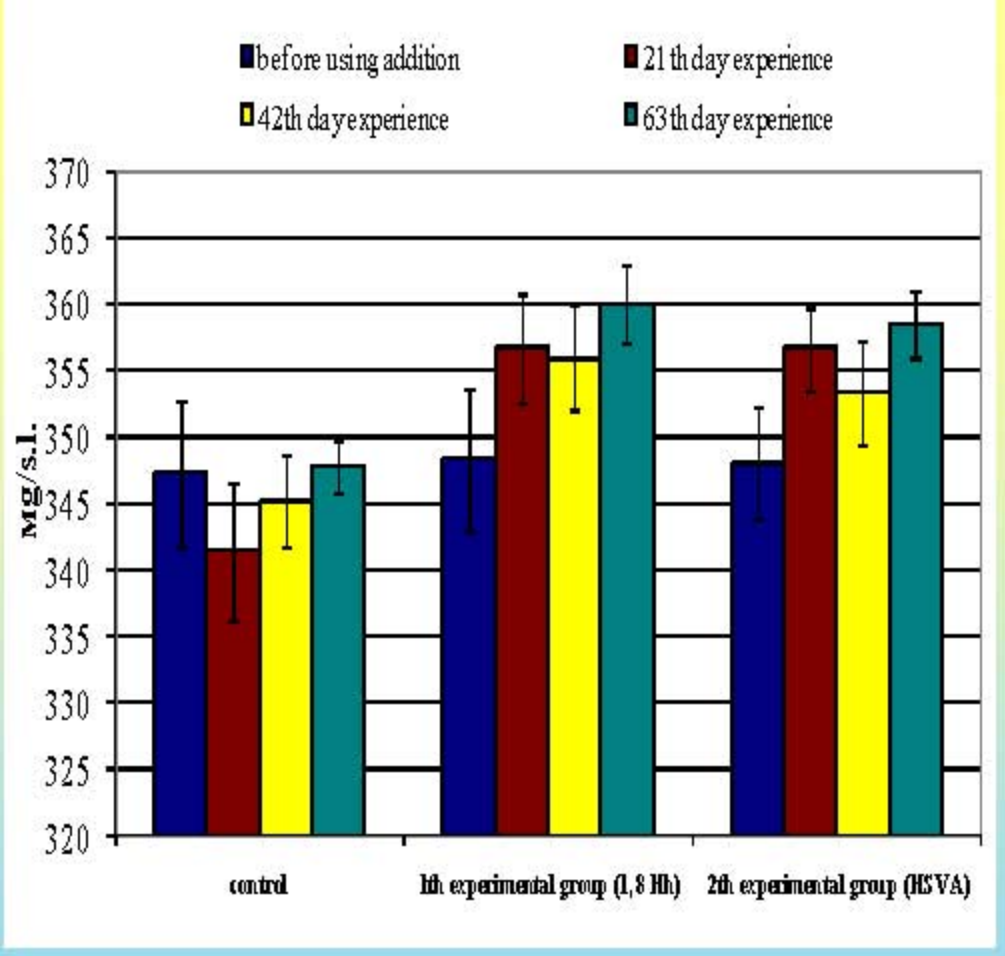
It can be assumed that humic substances enter into a living cell, participating in the process of transmembrane transport by the principle of intracellular digestion of the proposed de Dyuvom in 1964

In addition macromolecular products of intracellular digestion of HS with no peripheral components can communicate with both ions and free radicals more effectively than the native molecule of humic compounds, as in this case disappear steric hindrance for the contact.

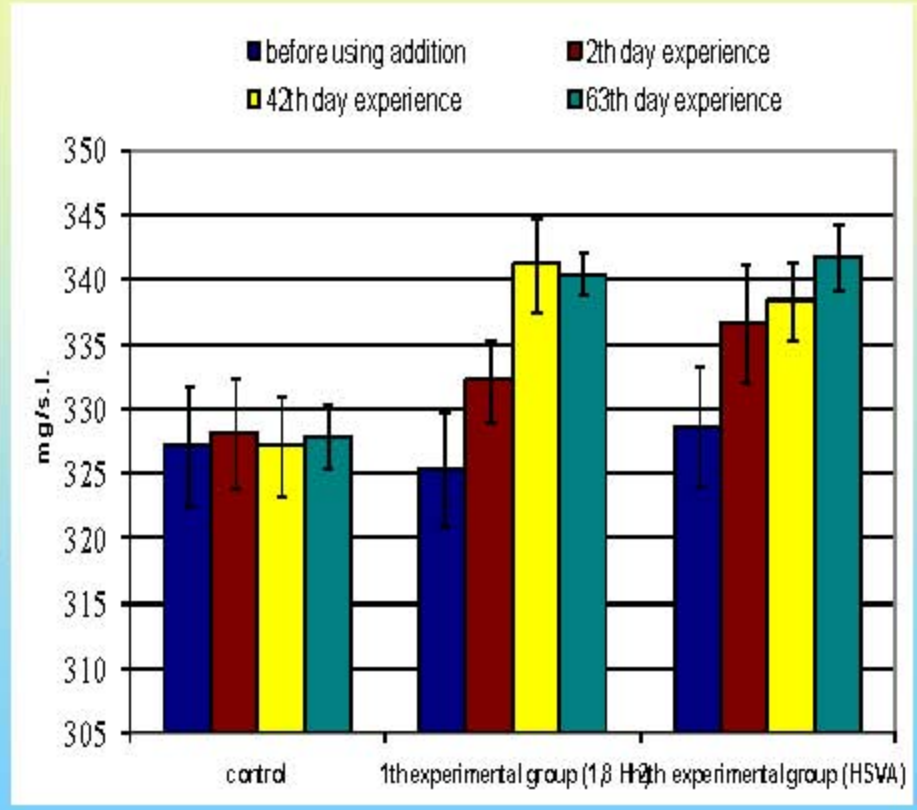
Proteoleticheskih dynamics of activity of enzymes of the mucous membrane of the duodenum and pancreas in poultry, (M±m, n=6)

During the experiment	Age, weeks	Group					
		Control			Experimental		
		chyme	mucous membrane	pancreas	chyme	mucous membrane	pancreas
before the use Hh	52	4,62 ± 0,6	3,76 ± 0,3	6,19 ± 0,4	4,49 ± 0,4	3,52 ± 0,6	6,48 ± 0,4
21-th (after fierst use Hh)	55	4,55 ± 0, 5	3,52 ± 0,3	5,55 ± 0,3	**6,33 ± 0, 6 ***	**5,73 ± 0,5 ****	**7,22 ± 0,3 ****
42-th	58	4,86 ± 0,3	4,53 ± 0,2	6,12 ± 0,5	**6,03 ± 0,5 ***	5,66 ± 0,5	**9,15 ± 1,0 ***
63-th (after the second use Hh)	61	4,68 ± 0,3	4,53 ± 0,2	6,63 ± 0,5	**7,48 ± 0,7 ****	**7,08 ± 0,3 *****	**8,73 ± 0,7 ***

note: * - p<0,05, ** - p<0,01, *** - p<0,001 to control



Activity amylase in duodenal contents of laying-hens
(M ± m, n = 6, мg/s.l.)

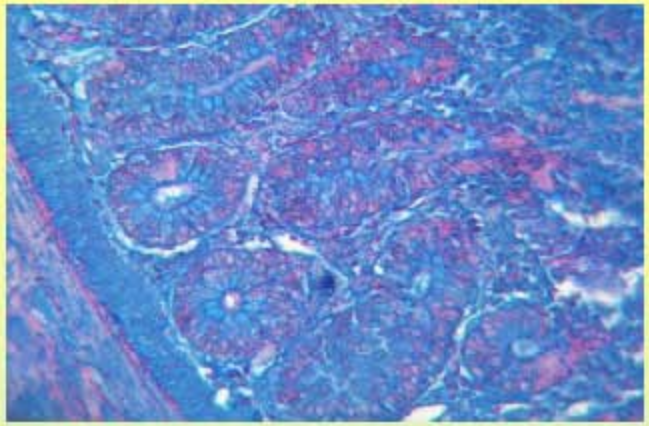


Activity amylase in12th intestinal mucosa of laying-hens,
(M ± m, n = 6, мg/s.l.)

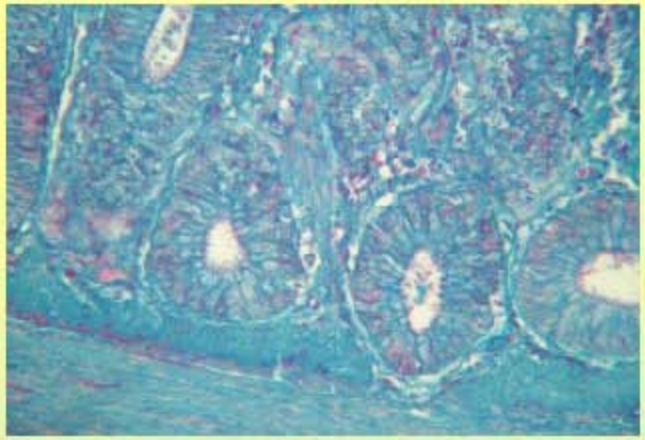
Amylase of the pancreas in poultry
(M ± m, n = 6, мg/s.l.)

Group	Period	Pancreas
Control group	1-th	421,03 ± 1,98
	21-th	419,25 ± 2,18
	42-th	416,44 ± 2,53
	63-th	418,74 ± 2,33
1th experimental group (Hh)	before using addition	415,68 ± 2,76
	21-th	**424,10 ± 1,12**
	42-th	**425,12 ± 1,09***
	63-th	**425,63 ± 1,05***
2 th experimental group (HSVA)	before using addition	416,19 ± 2,22
	21-th	**424,86 ± 0,86***
	42-th	**423,84 ± 1,55***
	63-th	**424,61 ± 0,94***

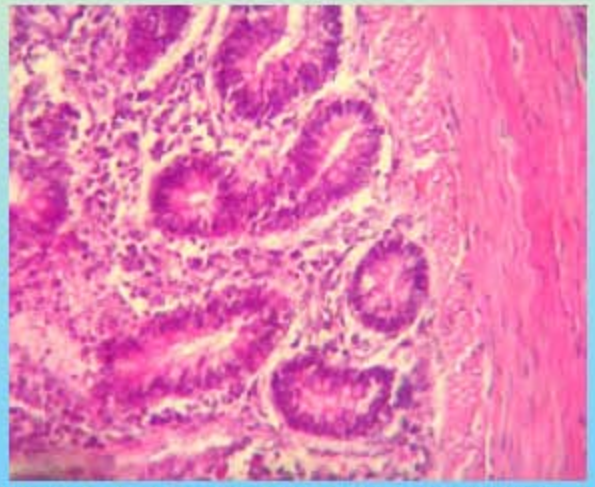
Note: * - p<0,05 to control group



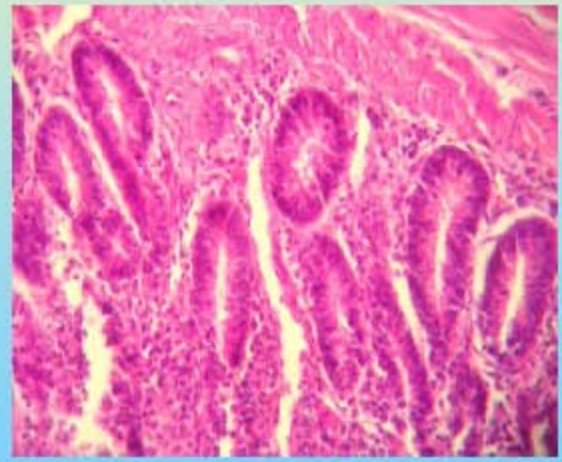
Histological preparations 12th intestine laying hens 63 days experiment after used Hydrohumate Гістопрепарат Заб. за Маллорі-Слінченко, ×400. Гіперплазія келихоподібних клітин у криптах: 1 – келихоподібні клітини; 2 – крипти.



Histological preparations 12th intestine laying hens 63 days experiment after used HSVA Гістопрепарат, Заб. за Маллорі-Слінченко, ×400. Гіперплазія келихоподібних клітин у криптах: 1 – келихоподібні клітини; 2 – крипти.

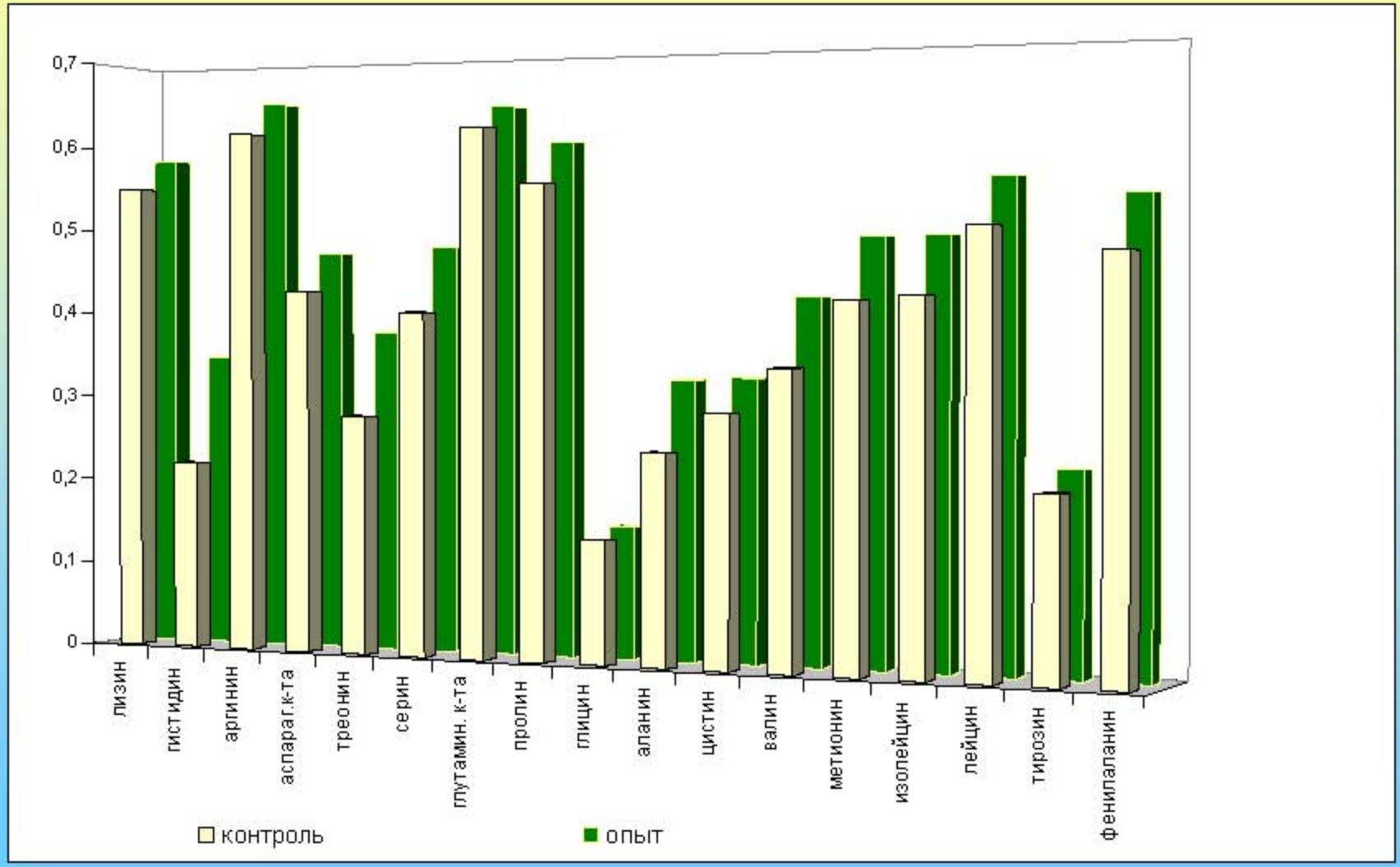


Histological preparations 12th intestine laying hens 63 days experiment after used Humate. Заб. гематоксиліном і еозином, ×400. Лейкоцитарна проліферація строми кишечнику.

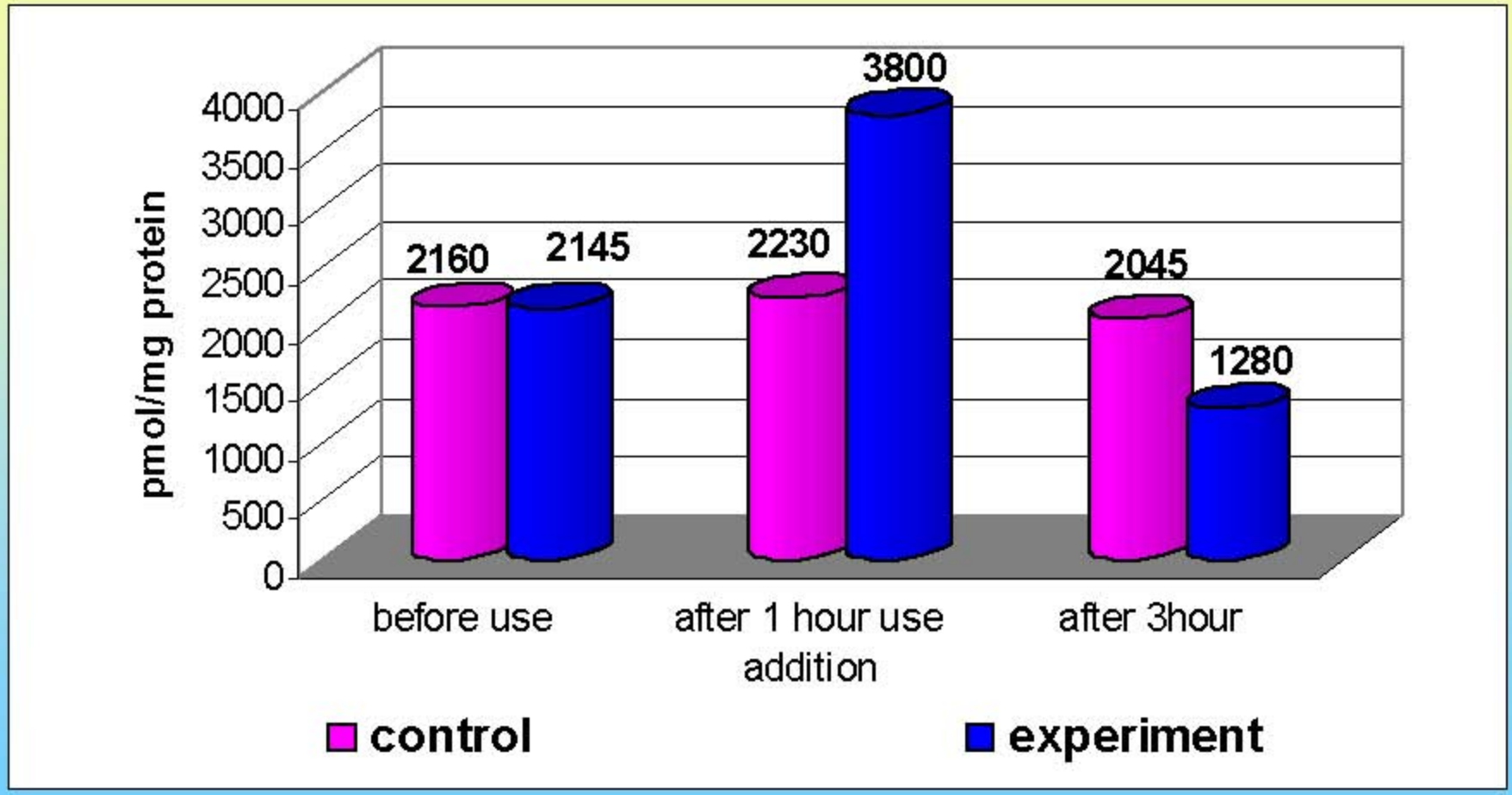


Histological preparations 12th intestine laying hens 63 days experiment after used HSVA, Заб. гематоксиліном і еозином, ×400. Лейкоцитарна проліферація строми кишечнику.

Assimilation acids in the organism under the influence of chickens Hydrohumate,%



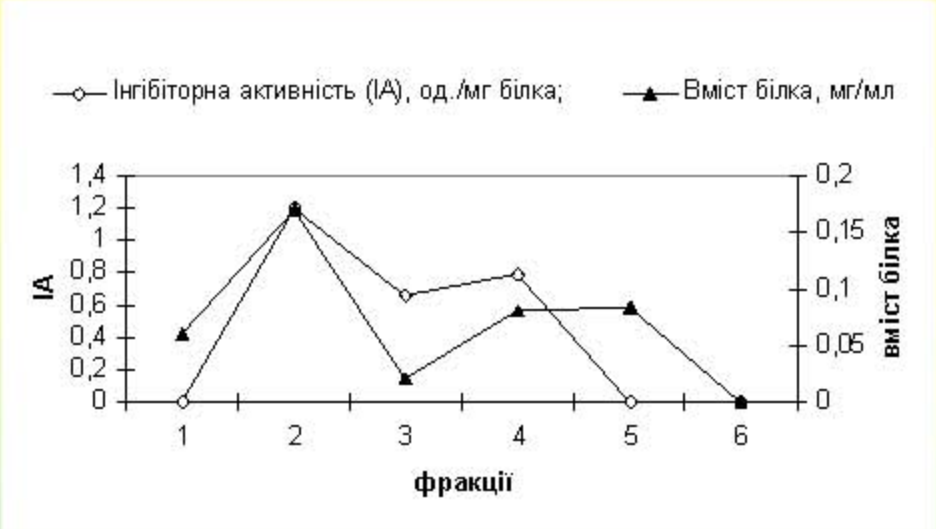
Addition influens on cAMP in in liver



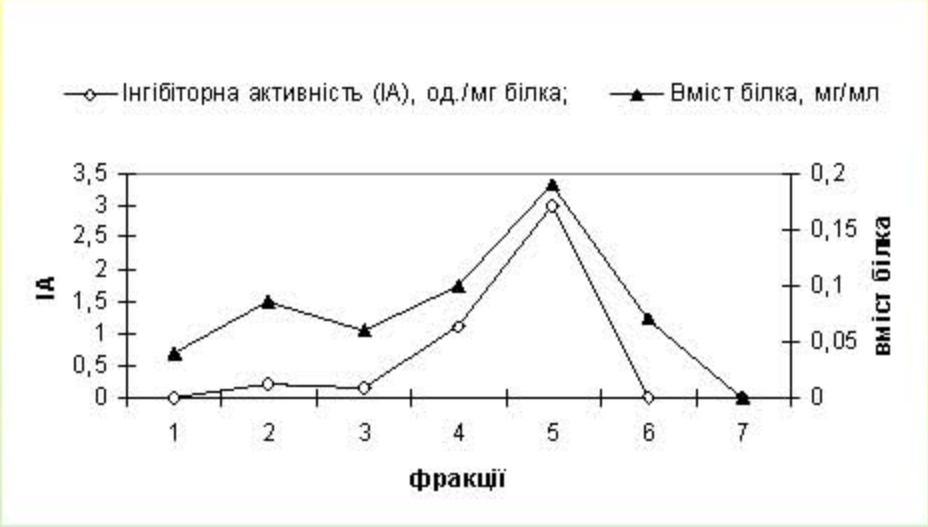
Activity katepsin B (mkg pNA/mg protein) and content protein (mg/ml) in extract fraction tissue liver of chicken under influence Hh (M±m, n=6)

Induces		Group	
		Control	Experiment
Extract	Protein	1,54+0,06	1,68+0,04*
	Activity	0,37+0,06	0,75+0,04***
Nuclear fraction	Protein	1,74+0,06	1,77+0,09
	Activity	0,34+0,03	0,84+0,05***
fraction of heavyweight mitochondriy	Protein	1,51+0,08	1,83+0,14
	Activity	0,36+0,07	0,74+0,16*
fraction of lightweight mitochondriy	Protein	0,01+0,001	0,02+0,004*
	Activity	0,35+0,04	0,78+0,20*
microsomal soluble fraction	Protein	1,49+0,07	2,24+0,09***
	Activity	0,36+0,06	0,81+0,08***

Note: * – $p<0,05$; ** – $p<0,01$; *** – $p<0,001$ to control.



A

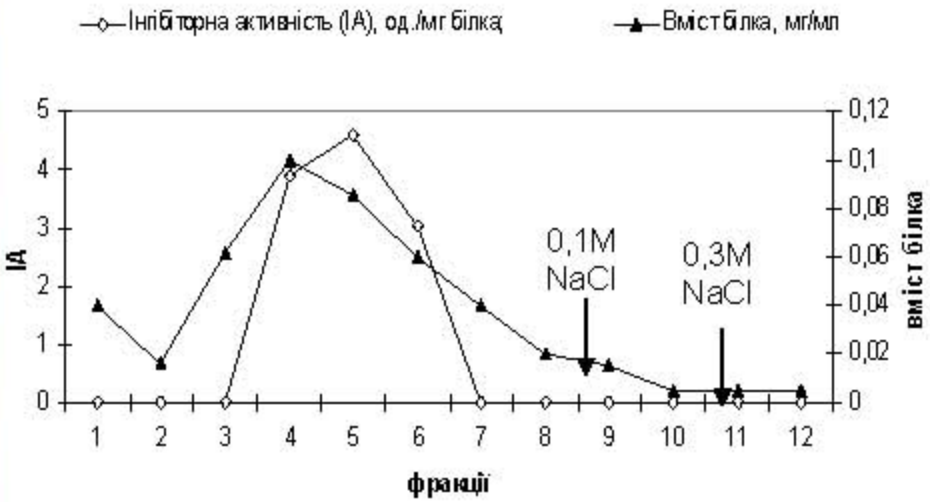


B

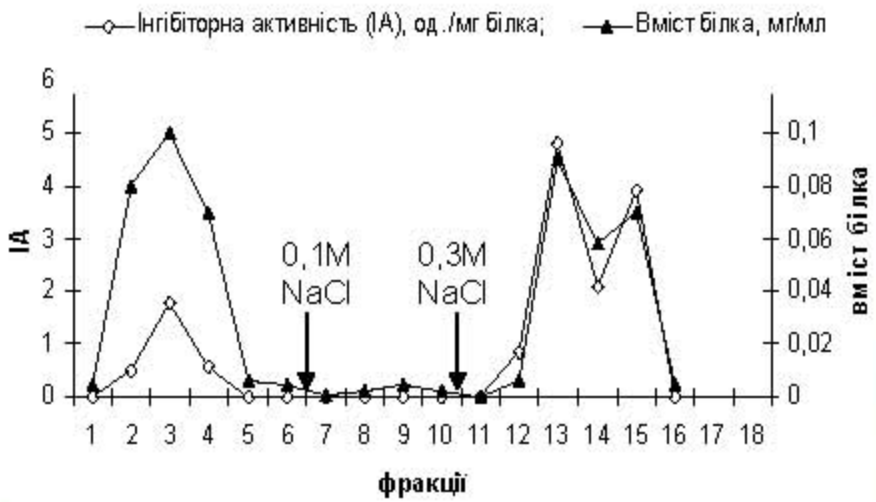
Gel chromatography on sephadex G-50 chicken liver extract control (A) and experimental (B) groups

By gel-filtration effluent inhibitory fraction of liver of broiler control and experimental groups on a column of Sephadex G-50 revealed a difference in the volumes of the fractions with inhibitory activity in partially purified enzyme preparation of cathepsin B

From the first fraction, which was derived from the liver of chicken-broiler control and experimental groups, as well as from the second fraction obtained from liver of chicken research, carried out further purification. Endogenous inhibitor was purified by ion-exchange chromatography on a column of DEAE Sephadex A-50. Proteins not associated with an ion exchanger, washed out 0.1 M Na-phosphate buffer (pH 6.0), which was balanced by a column. Elution of adsorbed protein was carried out stepwise application of buffer solutions with different ionic strength (0.1 M, 0.3 M NaCl) for more rapid and effective purification adsorbed inhibitor



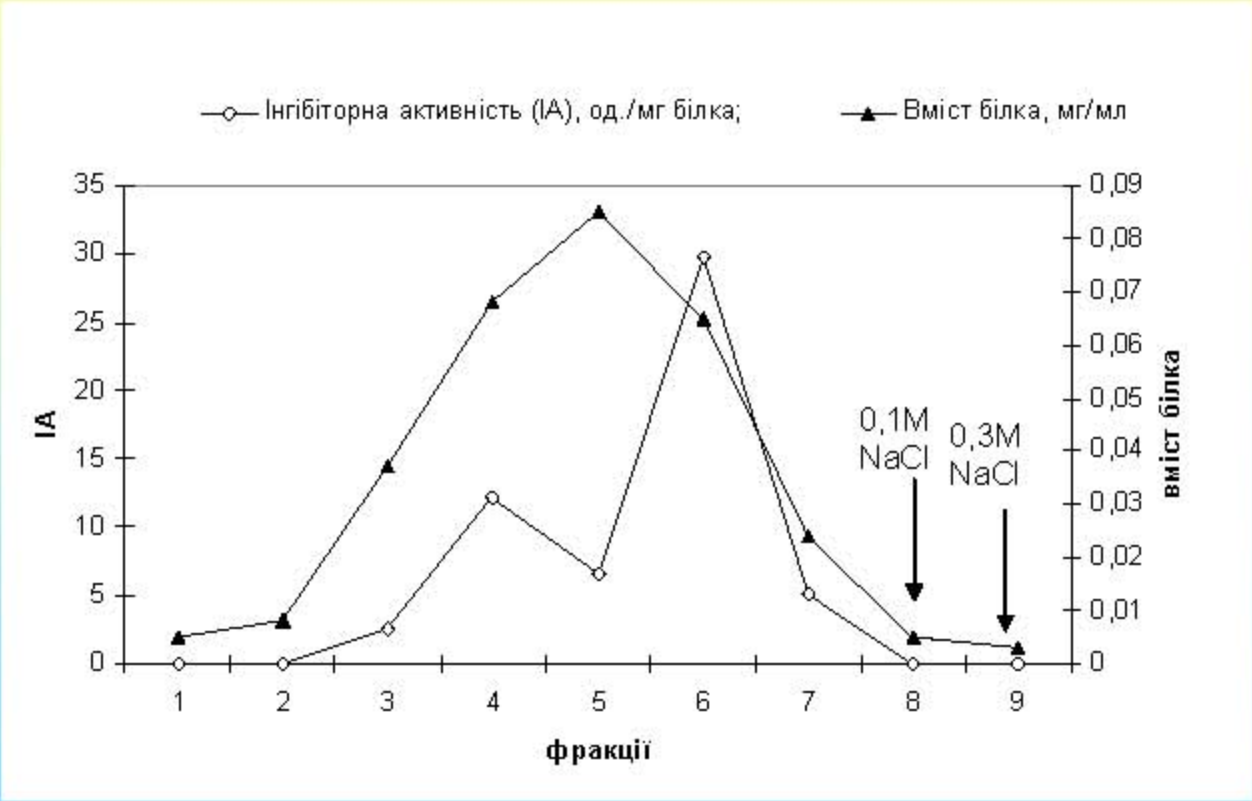
A



B

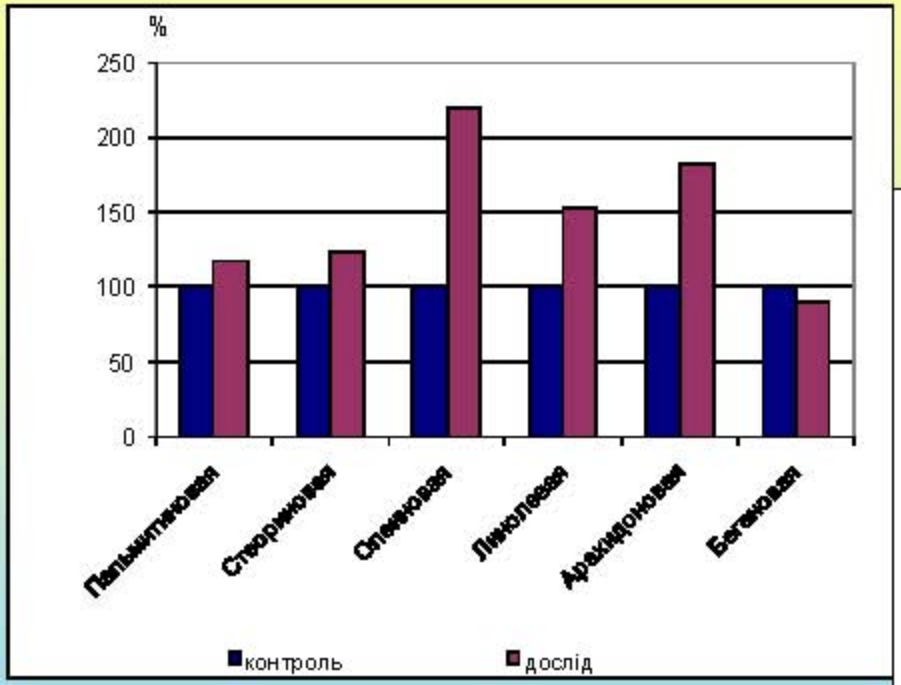
Ion-exchange chromatography on DEAE Sephadex A-50 first inigibitornoy fraction from the liver of broiler chickens of control (A) and experimental (B) groups

In the study of the second fraction, obtained from the liver of broiler-chickens in the experimental group was fixed by the main inhibitory activity, besides the protein is in the unbound state of the column with DEAE sephadex

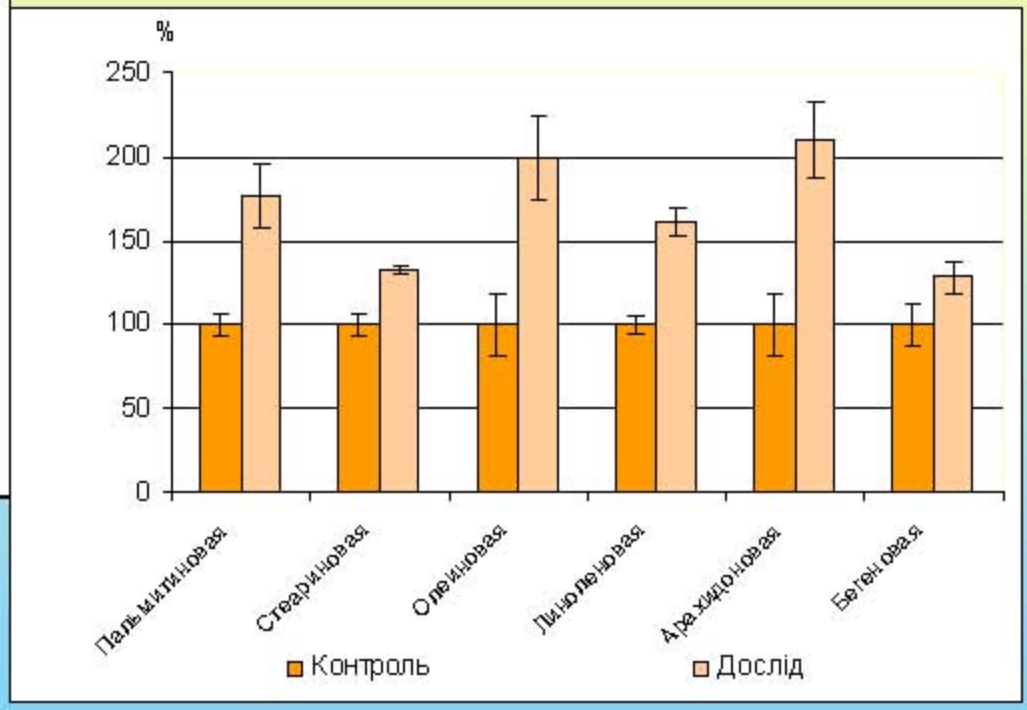


Ion-exchange chromatography DEAE Sephadex A-50 second inhibitory fraction from the liver of broiler-chickens of the experimental group

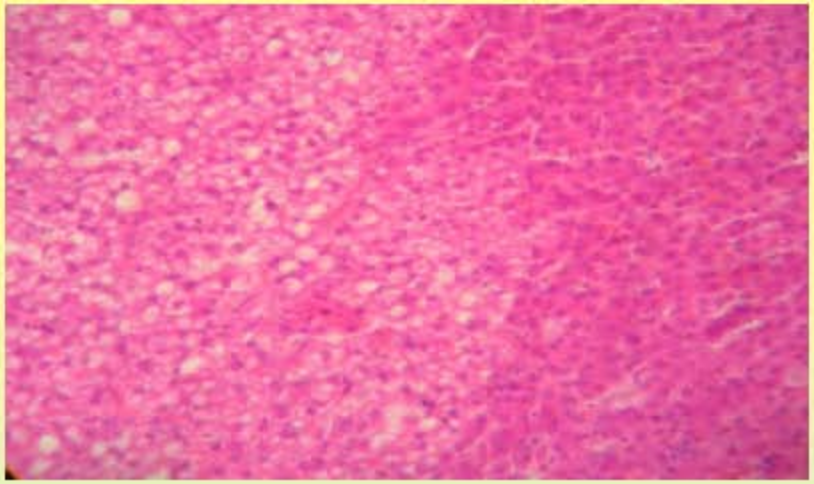
Free fatty acids in the liver tissue of chickens,% of control(M±m, n=10)



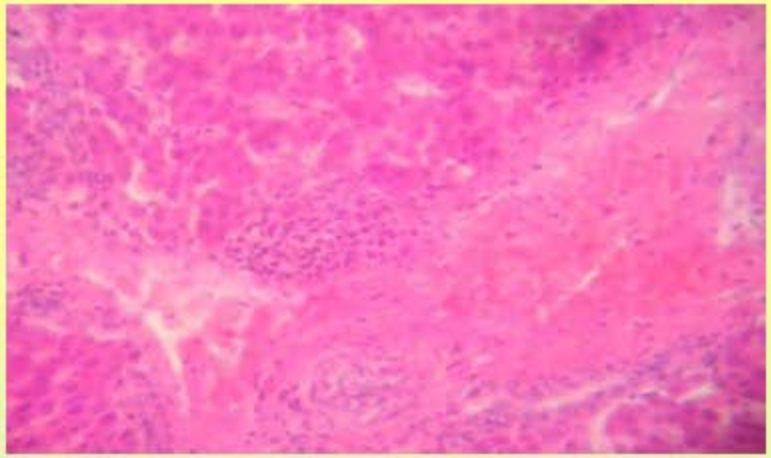
Before using hydrohumate



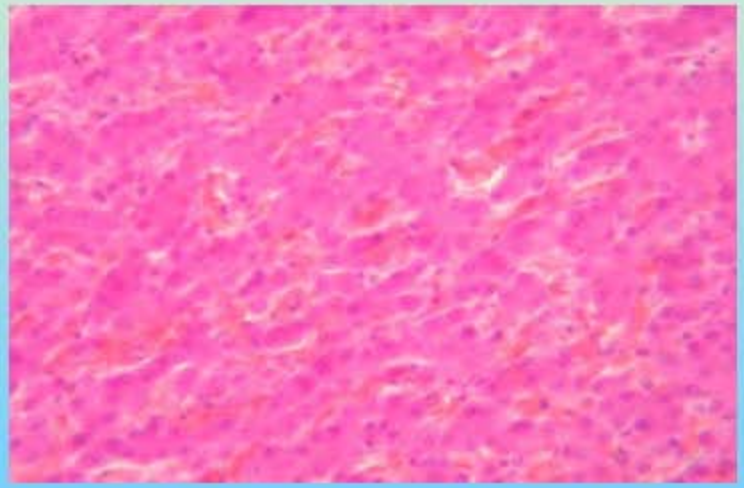
After using hydrohumate



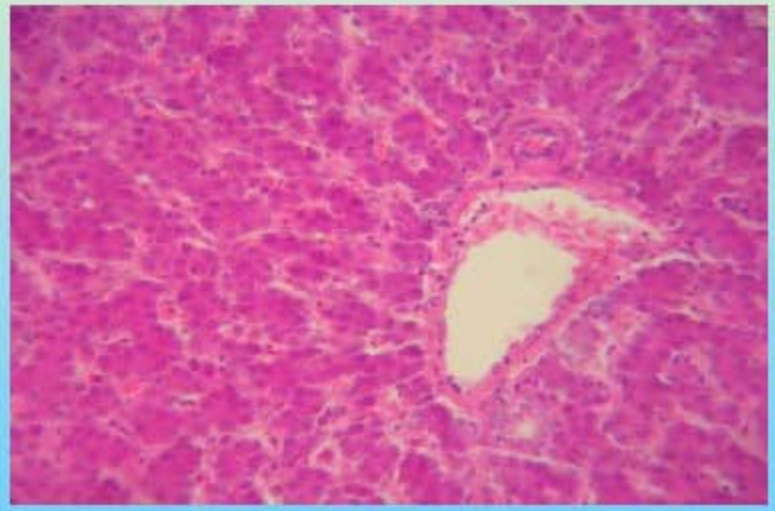
Histological preparations liver of laying-hens control groupe- 63 days experiment Заб. гематоксиліном і еозином, ×400. Осередок жирової інфільтрації гепатоцитів



Histological preparations liver of laying-hens control groupe- 63 days experiment after used influence humic additional Заб. гематоксиліном і еозином, ×400. Гіперплазія жовчного протоку та їх лімфоцитарна інфільтрація

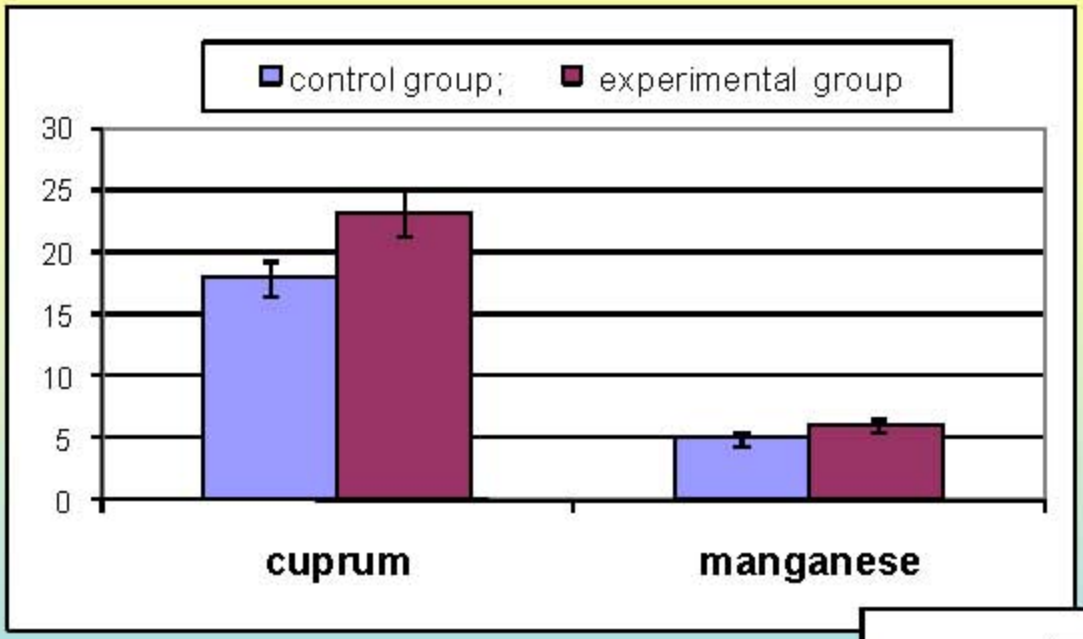


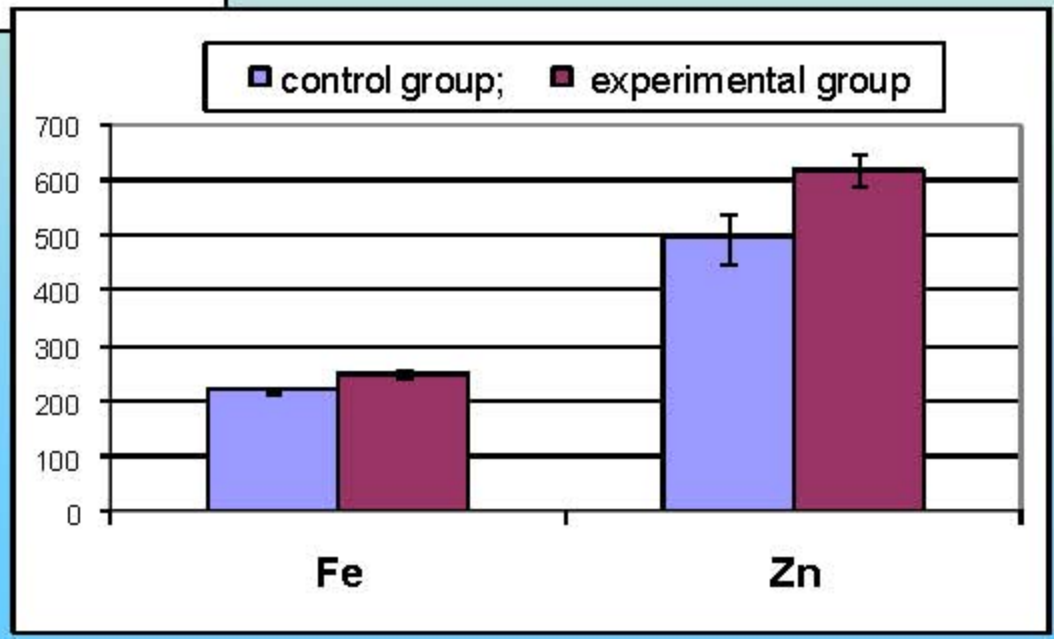
Histological preparations liver of laying-hens control groupe- 63 days experiment. Заб. гематоксиліном і еозином, ×400. Венозно-капілярне повнокров'я



Histological preparations liver of laying-hens control groupe- 63 days experiment Заб. гематоксиліном і еозином, ×400. Виражений поліморфізм ядер гепатоцитів, підвищений вміст двоядерних клітин

Microelement content in the liver of chicks under the influence Hydrohumate, **(M±m, n=4)**



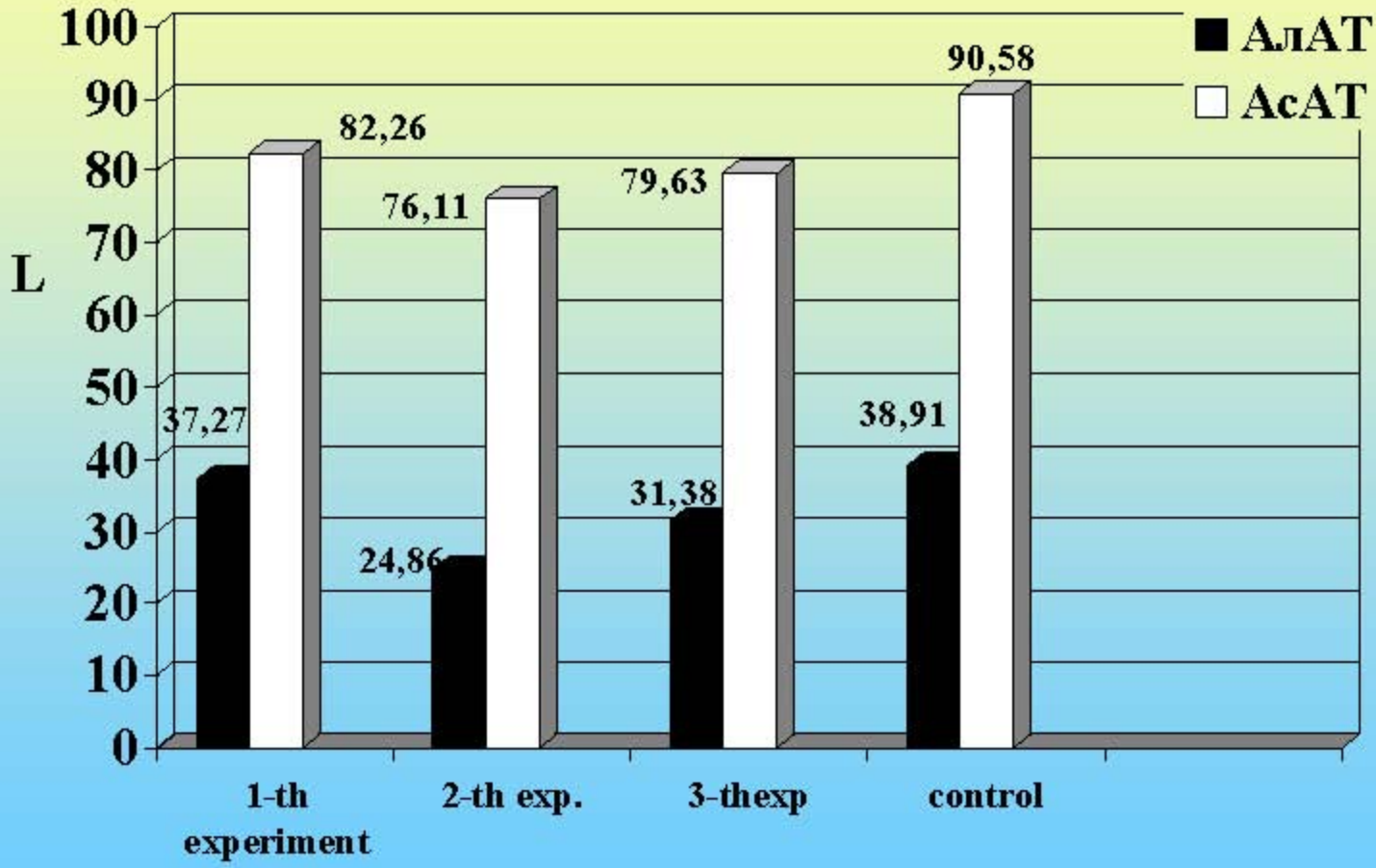


Activity of aspartate and alanine aminotransferase, gamma-glutamyl in blood laying hens under Hydrohumate(**ммol/(g•l), M±m, n=5**)

Groupe	Period	АсАТ	АлАТ	ГГТП
Control	1-th	0,85 ± 0,05	0,67± 0,07	66,40 ± 3,85
	21-th	0,87 ± 0,04	0,57± 0,05	68,60 ± 7,26
	42-th	0,84 ± 0,04	0,56 ± 0,04	67,80 ± 3,65
	63-th	0,86 ± 0,04	0,55 ± 0,03	66,80 ± 6,17
Hydrohumate	before using	0,83 ± 0,04	0,79 ± 0,12	66,20 ± 3,69
	21-th	**0,74 ± 0,04***	**0,86 ± 0,05****	**44,00 ± 2,83***
	42-th	0,76 ± 0,05	**0,78 ± 0,08***	56,00 ± 4,16
	63-th	**0,74 ± 0,02***	**0,79 ± 0,05****	**47,80 ± 4,58***

Note: * - p<0,05, ** - p<0,01 to control group

Transaminase activity of blood serum of calves to the combined effect of micronutrients and hydrohumate (n=7-10)

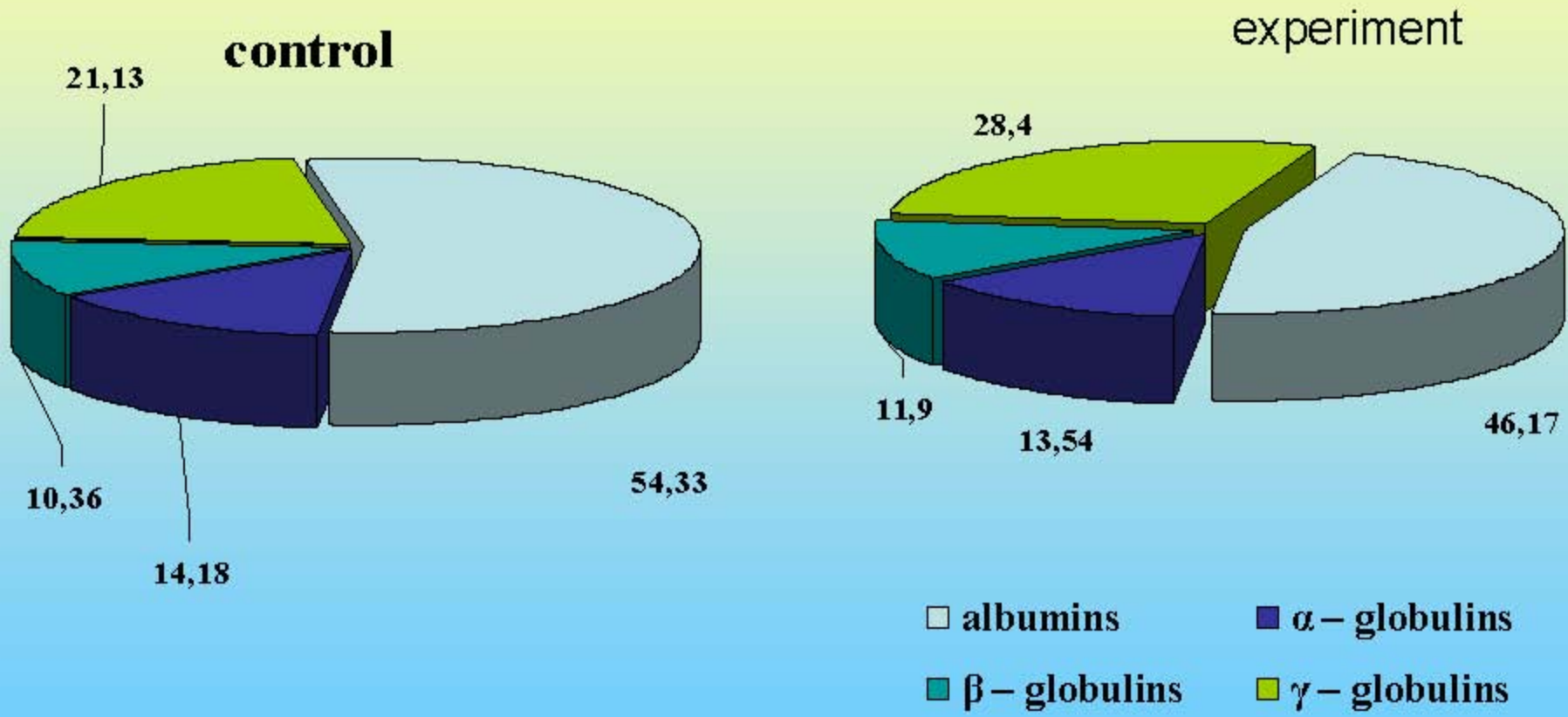


Total protein, albumin, globulin blood hens-layers under the influence Hh and HSVA (g/l, M ± m, n = 5)

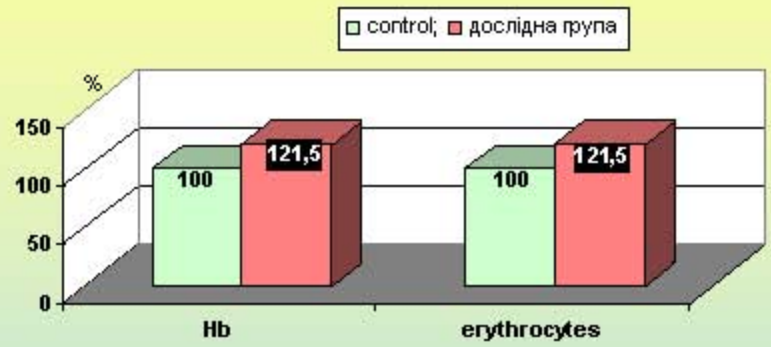
Група	Period	indices		
		Total protein	alb.	glob.
Control	1-th	53,46 ± 1,65	24,34 ± 0,85	29,12 ± 2,01
	21-th	49,00 ± 1,51	24,52 ± 1,92	27,30 ± 2,28
	42-th	44,06 ± 1,66	22,76 ± 1,63	21,30 ± 2,80
	63-th	42,06 ± 1,74	21,70 ± 1,19	20,36 ± 1,58
1th experiment (Hydrohumate)	before using	57,43 ± 1,95	25,90 ± 2,26	31,53 ± 2,17
	21-th	48,52 ± 2,26	**30,68 ± 0,59***	22,48 ± 1,88
	42-th	**51,00 ± 1,75***	23,68 ± 1,49	27,32 ± 1,52
	63-th	**54,46 ± 1,75****	**26,04 ± 0,90***	**28,42 ± 1,40****
2th experiment (HSVA)	before using	58,02 ± 1,14	26,36 ± 0,74	31,66 ± 1,31
	21-th	**57,86 ± 2,10****	27,46 ± 1,09	32,92 ± 2,63
	42-th	**52,20 ± 1,95***	23,30 ± 1,37	28,90 ± 3,10
	63-th	**56,12 ± 2,17*****	24,94 ± 1,08	**31,18 ± 2,60****

Note: p<0,05, ** - p<0,01, *** - p<0,001 to control

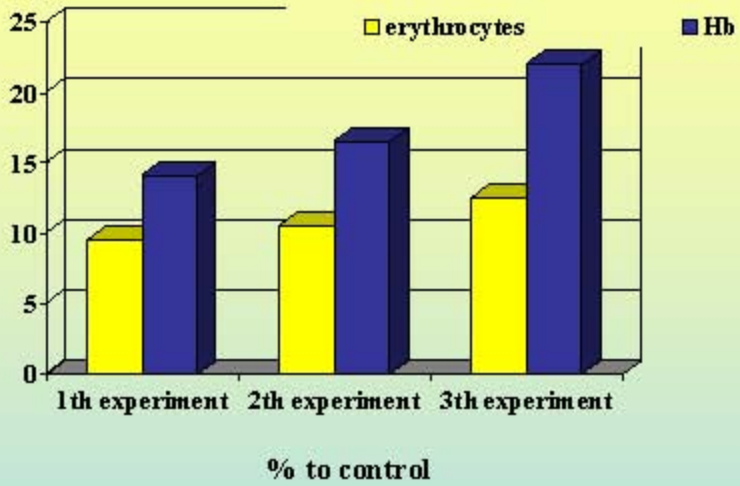
Percentage of protein fractions in blood serum of calves after a joint application Hydrohumate and microelements,%



Hydrohumate influence on morpho-functional blood ineices of laying hens



Influence Hydrohumate with combination microelements to the erythrocytes and hemoglobin in the blood of calves, (n=7)

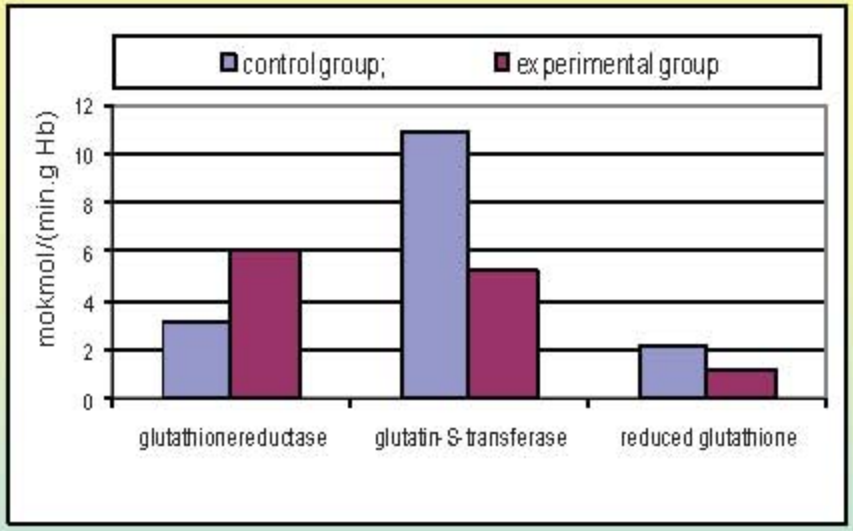


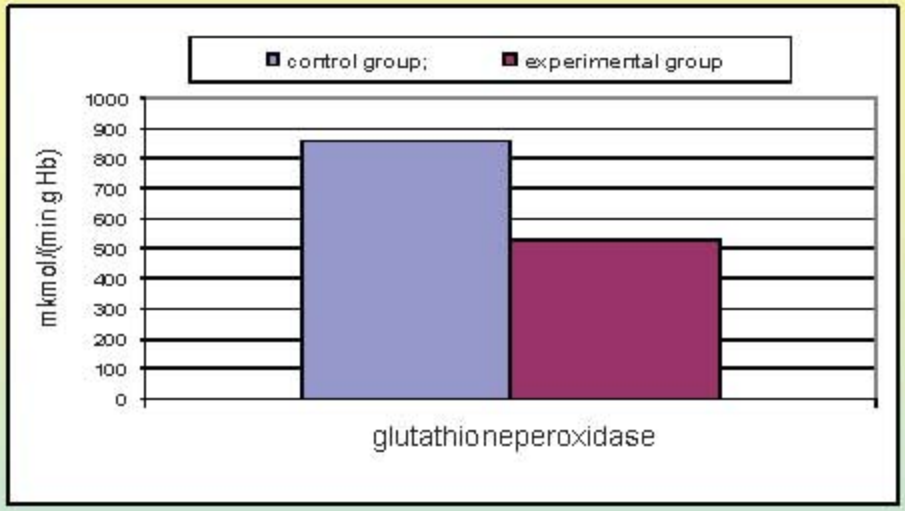
Hydrohumate influence on the index blood ostriches, M±m, n=6

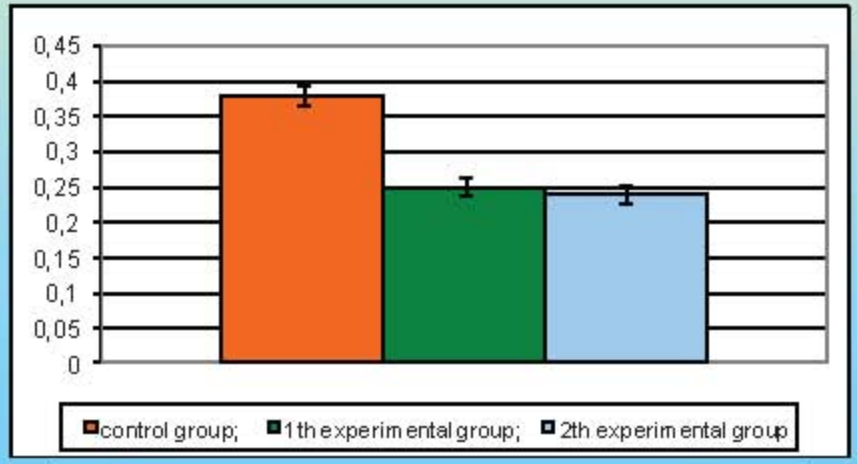
index	group	
	control	experience
Hemoglobin, g / l	111,50±3,36	171,33±3,48***
Erythrocytes, T/l	1,53±0,04	2,16±0,11***
Hematocrit, %	34±1,17	33,50±0,88

*- p<0,05; *** - p<0,001

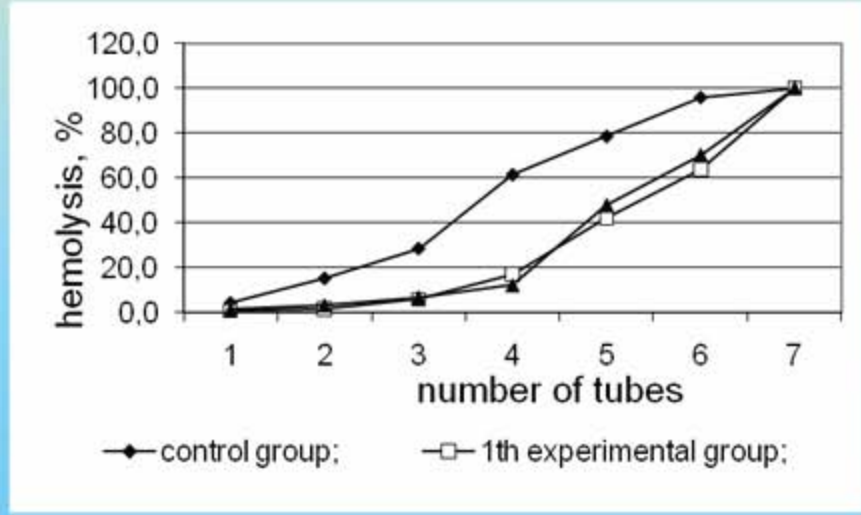
State of antioxidant protection eritrotsitov birds influenced Hydrohumate





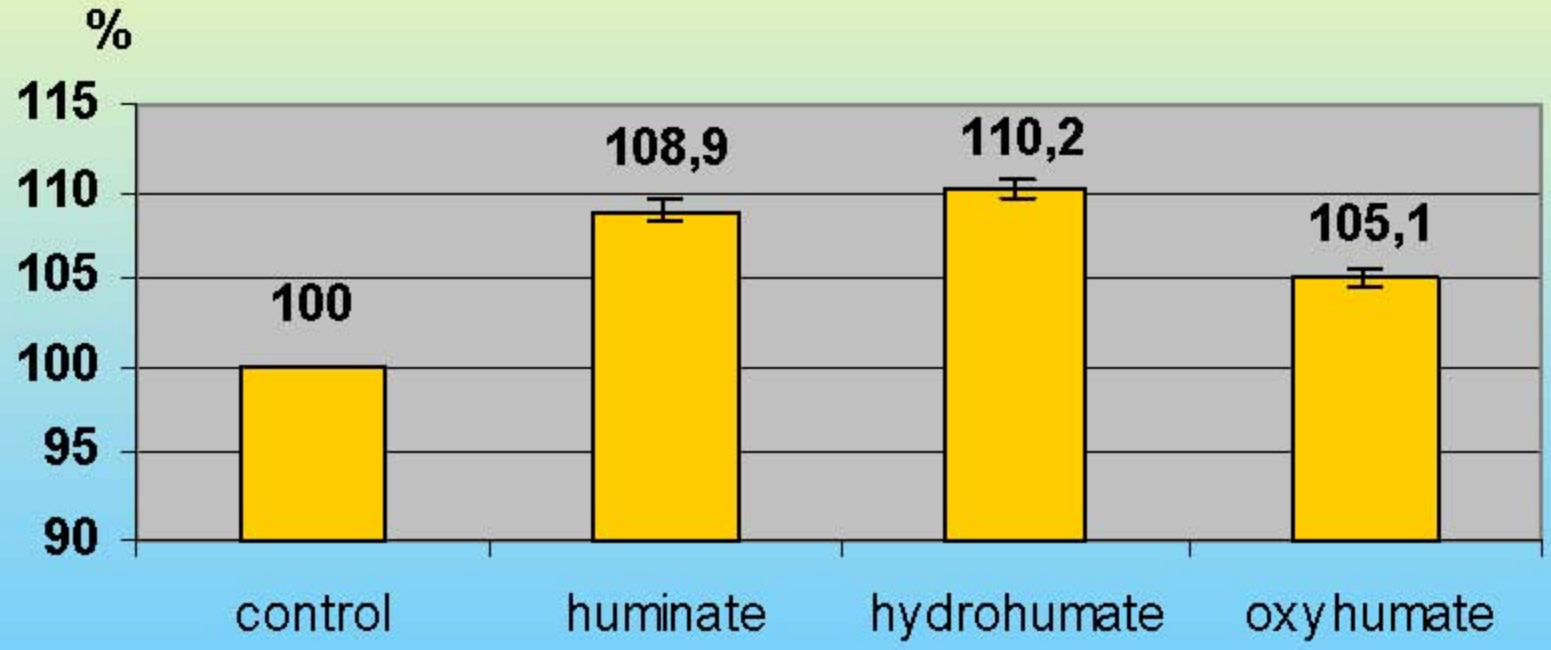


Content of TBA reactive products in erythrocytes blood of the poultry under the influence hydrohumate, мкмol/г Hb (M±m; n=6)



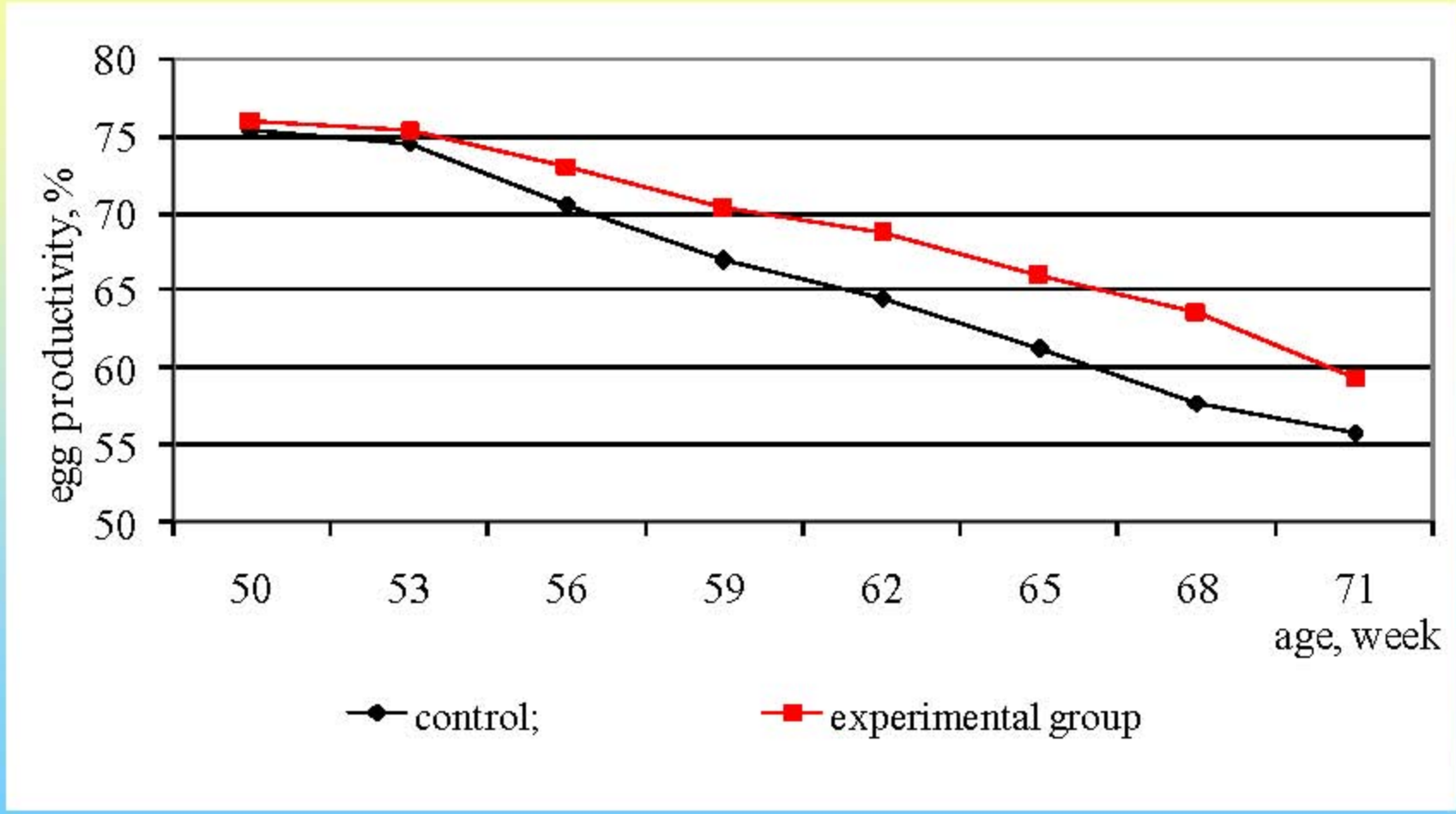
Curve of erythrocyte hemolysis layers under the influence of additives

Comparison of live weight of chickens under the influence of humic additions



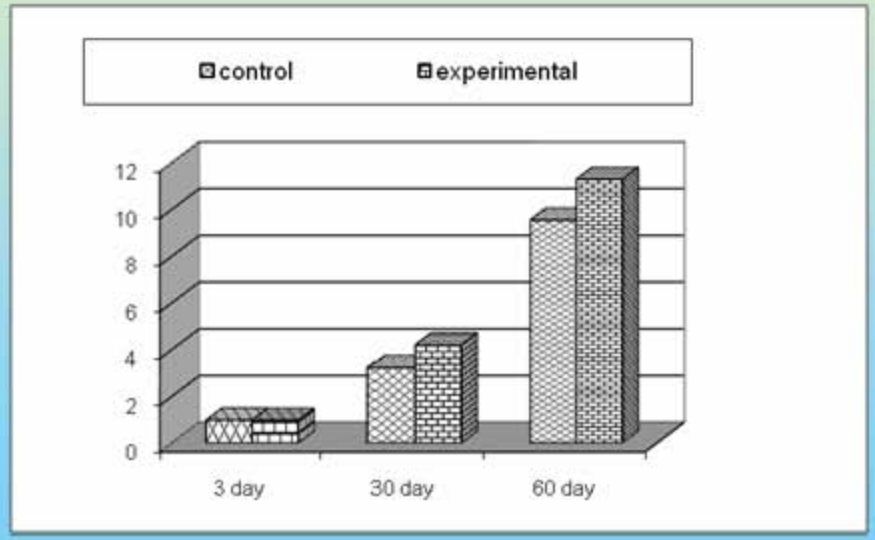
Average weight of chickens at the age of 44 days under using Humic additives, kg

Egg productivity of poultry under influence humic feed additives

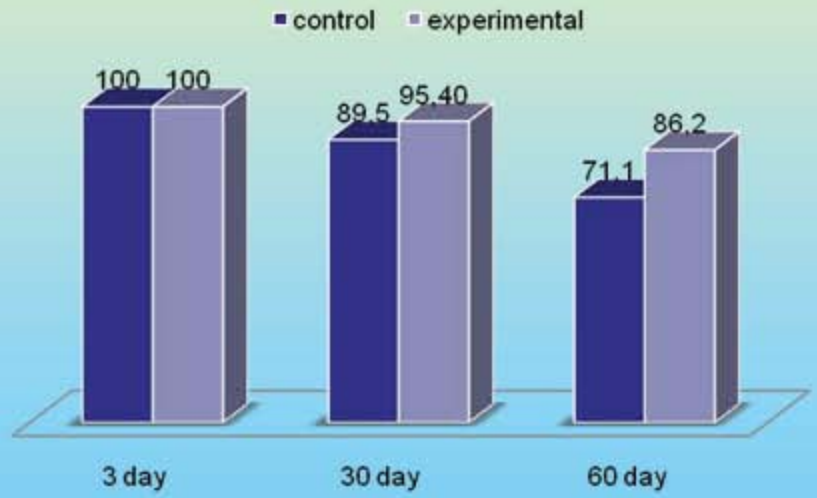


In the experiment, survival of a 30-day ostriches was 95.4% which is 6% exceeded the figure in control.

An ostrich's receiving hydrohumate for 60 days, the percentage of conservation was more of the control group at 15% and amounted to 86,2%. Weight strausyata experimental group at the age of 30 and 60 days exceeding the control by 24% and 30%.

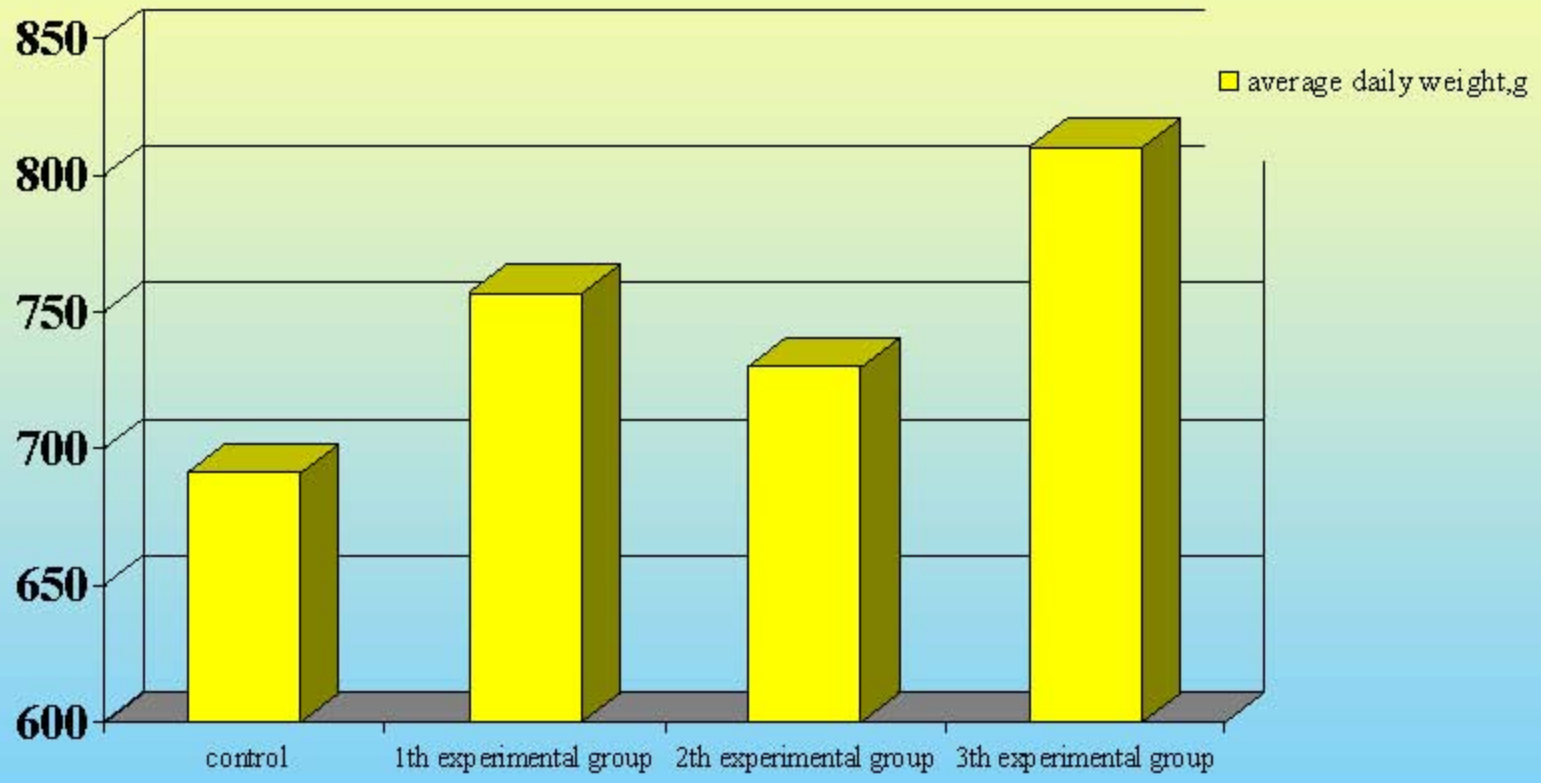


Average live weight under the influence of ostriches hydrohumate, kg



Survival under the influence of ostriches hydrohumate

Weight of calves under the influence of hydrohumate and trace elements



Concluding, humic substances of nature after the intake of animals can be included as a separate regulatory units in the complex system of hierarchical relationships, which is associated with their adjustment, immunomodulatory, antioxidant and antihypoxic effects.

Effect of feeding dietary supplements of humic nature of the organism of highly productive animals provides the transition of the organism to a new level gomeokinezu, which corresponds to higher rates of productivity.

History of studying the biological properties of humic substances is complicated enough. It can be distinguished from periods of high interest to her absence, or even a negative attitude towards this issue. Due to the fact that the interest in these issues has a discrete form, each return to the subject can give us new ways of solving this very difficult, but such a fascinating problem.


Thank you for your attention