UDC 612.014.46:636.52/.58

FUNCTIONAL STATUS OF ERYTHRON SYSTEM UNDER THE INFLUENCE OF T-2 TOXIN AND THE POSIBILITY OF CORECTION WITH AEROSIL

O. I. Fedyakova, I. Y. Kotsyumbas
olesja_v@ukr.net

State Scientific-Research Control Institute of Veterinary Preparations and Food Additives
Donetska str., 11, Lviv 79019, Ukraine

Often, changes of structural and functional state of erythron is a diagnostic indicator of the chemical factor influence on the body. Under the normal circumstances the red blood cells are able to partially counteract of various factors, but this resistance weakens with aging and negative factor's impact, including mycotoxins. Particularly dangerous (due to the wide share in nature) are the toxins of microscopic fungi from Fusarium family, including T-2 toxin, that belongs to the class 1 hazard (LD50 for most species in a single oral administration of 2–10 mg/kg. Since red blood cells are highly sensitive to chemical substances the morpholigical and functional *changes allow to set the intensity of toxic influence.*

The study was perfomed on Wistar male rats weighing 180–200 g. Two phases of research have been conducted to achieve this goal. The first one was study the effect of T-2 toxin after a single dose administration of 1/10 LD50. The effect of silica on day 4 after toxin administration was studied in the second phase.

The impact of T-2 toxin on certain haematological parameters of the rats' blood and erythron morphological and functional state was analysed. Depressive impact of T-2 toxin on the system of erythropoesis and protein biosynthesis was established, which leads to decrease in erythrocyte count, corpuscular volume haemoglobin concentration and derived indicators. Reduction in erythrocytes resistance to haemolytic and population redistribution of age fractions caused by accelerated cell aging under the influence of toxicant also found. The positive changes to all the above parameters during the simultaneous introduction of the toxin and silica was studied. Change of haematological parameters and dynamical reticulocytes exit indicates high absorption capacity of silica (aerosil) on T-2 toxin.

Keywords: ERYTHRON, T-2 TOXIN, HAEMATOLOGICAL PARAMETERS, RED BLOOD CELLS, HEMATOCRIT, HAEMOGLOBIN, ERYTHROGRAMS, FRACTIONATION OF ERYTHROCYTES

ФУНКЦІОНАЛЬНИЙ СТАН СИСТЕМИ ЕРИТРОНУ ЗА ДІЇ Т-2 ТОКСИНУ І МОЖЛИВІСТЬ КОРЕКЦІЇ АЕРОСИЛОМ

О. І. Федякова, І. Я. Коцюмбас
olesja_v@ukr.net

Державний науково-дослідний контрольний інститут ветеринарних препаратів та кормових добавок, 79019, м. Львів, вул. Донецька, 11

Часто зміни структурно-функціонального стану еритрона є діагностичним показником впливу хімічних факторів на організм. У нормальних умовах еритроцити здатні протидіяти в деякій мірі різним чинникам, але це знижує опір мембран. Такий ефект спостерігається при старінні і негативному впливі факторів, в тому числі мікотоксинів. Особливо небезпечні (через широкое поширення в природі) є токсини мікроскопічних грибів Fusarium, в тому числі Т-2 токсин, який належить до класу небезпеки 1 (LD50 для більшості видів в одному пероральному введенні 2–10 мг/кг. Оскільки

червоні кров'яні клітини дуже чутливі до хімічних речовин морфо-функціональні зміни дозволяють встановити інтенсивність токсичного впливу.

Дослідження було проведено на щурах-самцях лінії Вістар масою 180–200 г. Були проведені дві фази досліджень. По-перше, було вивчено вплив Т-2 токсину після одноразового введення дозою 1/10 ЛД50. Ефект аеросилу на 4-й день після введення токсину вивчали на другому етапі.

Досліджено вплив Т-2 токсину на окремі гематологічні показники крові щурів, морфо-функціональний стан еритрону. Встановлено депресивний вплив Т-2 токсину на систему еритропоезу та біосинтез білка, що призводить до зниження кількості еритроцитів, гематокритного числа, концентрації гемоглобіну та похідних показників. Виявлено також зниження стійкості еритроцитів до гемолітика та популяційний перерозподіл вікових фракцій зумовлений пришвидшеним старінням клітин під дією токсиканта. Вивчено позитивні зміни всіх вище зазначених показників при одночасному введенні з токсином аеросилу. Зміна гематологічних показників та динаміка виходу ретикулоцитів вказують на високі сорбційні здатності аеросилу щодо Т-2 токсину.

Ключові слова: ЕРИТРОН, Т-2 ТОКСИН, ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ, ЕРИТРОЦИТИ, ГЕМАТОКРИТ, ГЕМОГЛОБІН, ЕРИТРОГРАМИ, ФРАКЦІОНУВАННЯ ЕРИТРОЦИТІВ

ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ СИСТЕМЫ ЭРИТРОНА ПРИ ДЕЙСТВИИ Т-2 ТОКИНА И ВОЗМОЖНОСТЬ КОРРЕКЦИИ АЭРОСИЛОМ

А. И. Федякова, И. Я. Коцюмбас
olesja_v@ukr.net

Государственный научно-исследовательский контрольный институт ветеринарных препаратов и кормовых добавок, 79019, г. Львов, ул. Донецкая, 11

Часто изменения структурно-функционального состояния эритрона является диагностическим показателем воздействия химических факторов на организм. В нормальных условиях эритроциты способны противодействовать различным факторам, но это снижает сопротивление мембран. Такой эффект наблюдается при старении и негативном воздействии факторов, в том числе микотоксинов. Особенно опасны (из-за широкой распространенности в природе) являются токсины микроскопических грибов Fusarium, в том числе Т-2 токсин, который принадлежит к классу опасности 1 (LD50 для большинства видов в одном пероральном введении 2–10 мг/кг. Поскольку красные кровяные клетки очень чувствительны к химическим веществам морфо-функциональные изменения позволяют установить интенсивность токсического воздействия.

Исследование было проведено на крысах-самцах линии Вистар массой 180–200 г. Были проведены две фазы исследований. Первым было изучено влияние Т-2 токсина после однократного введения в дозе 1/10 ЛД50. Эффект аеросила на 4-й день после введения токсина изучали на втором этапе.

Исследовано влияние Т-2 токсина на отдельные гематологические показатели крови крыс, морфо-функциональное состояние эритрона. Установлено депрессивное влияние Т-2 токсина на систему эритропоэза и биосинтез белка, что приводит к снижению количества эритроцитов, гематокритного числа, концентрации гемоглобина и производных показателей. Выявлено также снижение устойчивости эритроцитов к гемолитику и популяционное перераспределение возрастных фракций, которое обусловлено ускоренным старением клеток под действием токсиканта. Изучены положительные изменения всех вышеуказанных показателей при одновременном введении с токсином аэросила. Изменение гематологических показателей и динамика выхода ретикулоцитов указывают на высокие сорбционные способности аэросила относительно Т-2 токсина.

Ключевые слова: ЭРИТРОН, Т-2 ТОКСИН, ГЕМАТОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ, ЭРИТРОЦИТЫ, ГЕМАТОКРИТ, ГЕМОГЛОБИН, ЭРИТРОГРАММЫ, ФРАКЦИОНИРОВАНИЕ ЭРИТРОЦИТОВ

Often, changes of structural and functional state of erythron is a diagnostic indicator of chemical factors influence on the body. As a result, there is a damage to cell membranes and related interruption in bio-energetic processes. Under the normal circumstances the red blood cells are able to oppose at some degree of various factors, but this resistance weakens with aging and negative factor's impact, including secondary metabolites of mould fungi – mycotoxins.

Particularly dangerous (due to the wide share in nature) are the toxins of microscopic fungi from Fusarium family [1, 2]. 45 of tryhothecenes mycotoxins are among the most dangerous secondary fungi metabolites, including T-2 toxin, that belongs to the class 1 hazard (LD50 for most species in a single oral administration of 2–10 mg/kg [3]). Observed effects under the influence of T-2 toxin are: dermatonecrosis, lesions of the cardiovascular system, gastrointestinal tract, decreased animal productivity and resistance to diseases [4]. The negative impact of T-2 toxin on the functioning of the haepathobiliary [5] system and bone marrow are well known [6], however, the influence at the blood system and the prevention of this phenomena studied insufficiently. In this respect, worthwhile is the study not only about the haematological parameters, but also the age of the erythrocyte membrane and erythrocytes resistance.

To prevent the negative effects of feeding nutriments contaminated by mycotoxins, a wide range of feed additives such as enterosorbents are used. They are an effective treatment and prophylactic measure of various etiologies. Enterosorbents are introduced into the nutriments during most of the period during breeding animals, therefore they must meet the following requirements: be effective when introducing only a few kg/t; bind toxins at low concentrations; be safe and keep up the growth rates of animals. Aerosil is one of the most effective sorbents on tryhothecenes mycotoxins. It meets all the above requirements and eliminates the T-2 toxin by sorption on molecule's surface and elimination from the body

Since red blood cells are highly sensitive to chemical substances and morpholigical and functional changes allow set the intensity of toxic influence – the purpose of our study was to determine the impact of T-2 toxin on haematological parameters in rats and to investigate the state of erythron with using of aerosil.

Materials and Methods

The study was perfomed on Wistar male rats weighing 180–200 g that were kept isolated (on quarantine) for 7 days before the experiment. According to the principle of analogues there were 2 control and 7 experimental groups of 5 animals each formed.

Two phases of research have been conducted to achieve the goal. The first one was study the effect of T-2 toxin after a single dose administration of 1/10 LD50. In order, to do this rats were divided into 6 groups: control (K) and 5 experimental (D1-D5). Experimental groups were administered T-2 toxin intragastrically, through the probe, at a dose of 0.67 mg/kg. The effect of silica on day 4 after toxin administration was studied in the second phase. Three groups of animals were formed: Control — K2; animals injected with T-2 toxin — D6; animals injected with T-2 toxin along the silica at the rate of 200 mg/kg body weight — D7.

Blood was selected after animal decapitation under a light ether anaesthesia (in compliance with the European Convention for the protection of laboratory animals used in the experiment) and the following parameters defined: haemoglobin concentration [7], the number of erythrocytes, and reticulocytes [8], hematocrit, acid resistance of erythrocytes [9], the erythrocytes composition component also determined in sucrose density gradient. Cell

populations in the age aspect obtained for differentiation, cytological control of the reticulocytes content [10] was performed. The mean concentration of haemoglobin and the mean volume of red blood cells were defined by calculation. Software package Microsoft Office Excel 2010 was used for statistical analysis of the received results.

Results and discussion

The experimental data showed that a single intragastric administration of T-2 toxin at the dose 1/10 LD50 resulting in credible changes in some haematological indicators, reveal a malfunction of erythron.

In particular, during the first day of the experiment, decrease in haemoglobin concentration by 11 % was observed, and on day 3 of the experiment it was 103.29 g/L, and by the 5th day there were no significant changes in comparison with control.

Reduction in the number of red blood cells during the experimental period was less expressed, however on day 4 of the experiment, the number was 4.36 t/l. These changes are generated by accelerating destruction of cells in the bloodstream, caused by haemolysis due to oxidative stress triggered by T-2 toxin.

Hematocrit number is changing similar to the number of red blood cells (starts to decrease straight in the first days after toxin administration) and reaches the maximum alteration, namely 43.13 % on the 4th days of the experiment. On the 5th day after administration of toxin this number is starting to the control group and stands at 48.13 %.

The morphological and functional changes in the hematopoietic system of rats (during development of toxicosis) are indicated by the research results of mean erythrocyte volume, and concentration of haemoglobin in erythrocyte. Despite the fact that mentioned numbers are derived from hematocrit, erythrocyte count and haemoglobin concentration in the blood, their calculation is often carried out in research for more complete characteristics of the processes occurring in the system of erythropoiesis influenced by various factors.

The results of our research indicate that the introduction of T-2 toxin causes erythrocyte volume to increase, so on days 1–4 of the experiment, this index increases by 7–13 %. This fact, as known, is typical for haemolytic and other anaemia, and also disfunction of liver.

The mean concentration of haemoglobin in erythrocytes indicator shows the saturation level of erythrocyte by molecules of oxygen-transport protein. Although it is the most stable haematological parameter it was established that on day 4, after toxin administration, it increased by 10 %, and on day 5 approximate control values. These data indicate abnormal synthesis of haemoglobin under the toxin influence.

Table 1

Influence of T-2 toxin on hematological parameters of blood in rats

	Hemoglobin, g/l	Red blood cells, T/l	Hematocrit, %	Mean corpuscular volume, fl	Mean corpuscular Hgb, pg	Mean corpuscular Hgb concentration
K1 (n=25)	131.85±6.86	5.94±0.17	51.87±0.52	87.32	22.2	3.93
D1 (n=5)	118.07±6.86	5.36±0.18*	50±0.85	93.28	22.03	4.23
D2 (n=5)	113.14±4.33	5.19±0.22*	48.63±0.52**	93.69	21.8	4.3
D3 (n=5)	103.29±3.92*	4.48±0.23***	44.0±0.74***	98.21	23.06	4.26
D4 (n=5)	107.03±6.53*	4.36±0.21***	43.13±0.36***	98.92	24.55	4.03
D5 (n=5)	122.38±5.38	5.42±0.21	48.13±0.32***	88.8	22.58	3.93

Note: * — $p<0.1$; ** — $p<0.01$; *** — $p<0.001$ compared with a control group

Having analyzed the acid resistance of erythrocytes, the statement can be made, that maximum resistance is observed in the blood of the control group animals (K1), as can be seen from the graph left and right sides of peak are symmetrical, indicating a balance between young, mature and old red blood cells. Similar resistance was found in samples of the first (D1) and fifth (D5) groups, however, in the samples of group 1, increase in old and decrease in young erythrocytes was observed, as indicated in the left graph, which is much bigger than the right. These changes are caused by inhibition of erythropoiesis due to depressive effect of the toxin on the blood. Decreased resistance of red blood cells due to a slowdown in the synthesis of new cells, was observed in erythrograms D2 and D3 groups, the peak which is shifted to the left in comparison with the control ($p<0.1$). In group D4 the greatest resistance of erythrocytes was noted. The peak maximum appeared at 4.5 min and contained 43 % haemolysed cells. These changes, evidently, were caused by activation of lipid peroxidation in these cells, and by the development of oxidative stress leading to cell aging. Analyzing data on day 5 after administration of toxicant, maximum haemolysis observed at 5.5 min. This indicates the normalization of erythropoiesis intensity, the functional cells condition, and increased resistance of erythrocytes to the haemolytic action, as evidenced by the expansion of the right part of the graph.

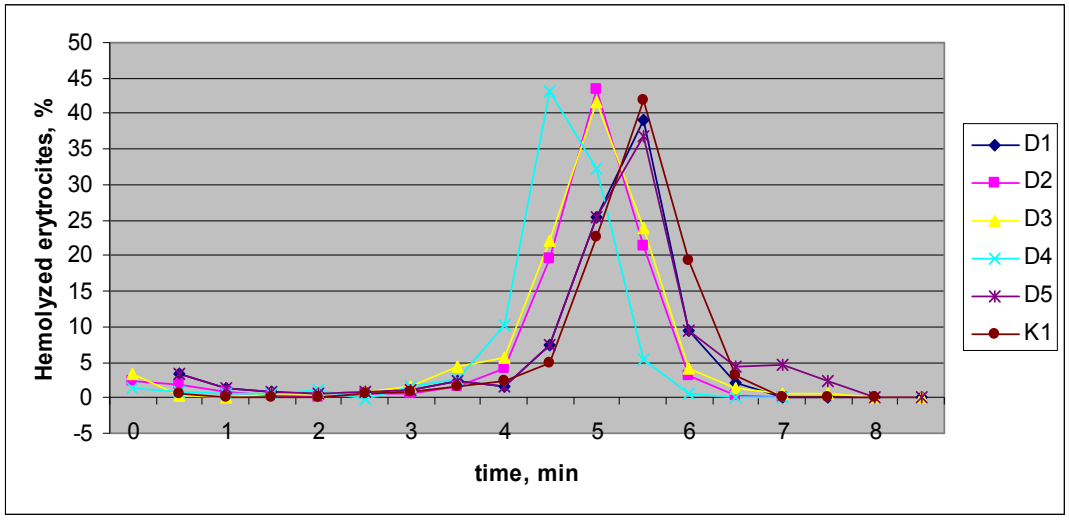

Fig. 1. Influence of T-2 toxin on acid resistance of rat erythrocytes

So, the results obtained at this stage of research, pointing at the negative impact of T-2 toxin on the system of erythropoiesis which is manifested in changes of haematological parameters. Reduction in the number of red blood cells and haemoglobin concentration in the animal blood under the influence of the tested toxicant reduces the oxygen supply to the tissues. It is likely that the number of red blood cells decreases due to interruption in the processes of erythropoiesis in the marrow and the growth rate of destruction of red blood cells in the spleen. In general, this impact of T-2 toxin indicate suppression of erythropoiesis and accelerated destruction of erythron cells due to oxidative stress.

From the results obtained it can be concluded that the maximum change in haematological parameters in rats was observed on the 4th day after administration of T-2 toxin, therefore, to study the impact of neutralizing effect of aerosil in the next stage of our work we have chosen this period.

Experiments were performed on three groups of animals: K2 (control), D6, D7 (group of animals injected with T-2 toxin), D7 (animals which additionally were injected the aerosil).

Data analysis showed neutralizing effect of silica on toxin action. The samples of D7 group showed the number of erythrocytes increase by 14.5 % compared to the D6 group animals ($p<0.1$), injected with toxin.

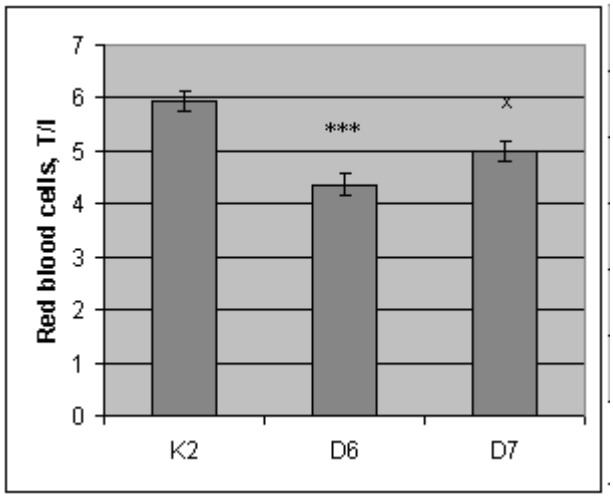

Fig. 2. Quantity of red blood cells in the blood of rats by administration of T-2 toxin and aerosil
Note: x — $p<0.1$; xx — $p<0.01$; xxx — $p<0.001$ compared with a group D6

By the action of T-2 toxin in animals of the experimental group (D6) significant ($p<0.1$) decrease in haemoglobin concentration by 19 % compared with control (K2) occurred. This may be due to inhibition of protein synthesis of T-2 toxin. Given silica growing this figure by 6 %, but these changes are not credible when compared with animals of D6.

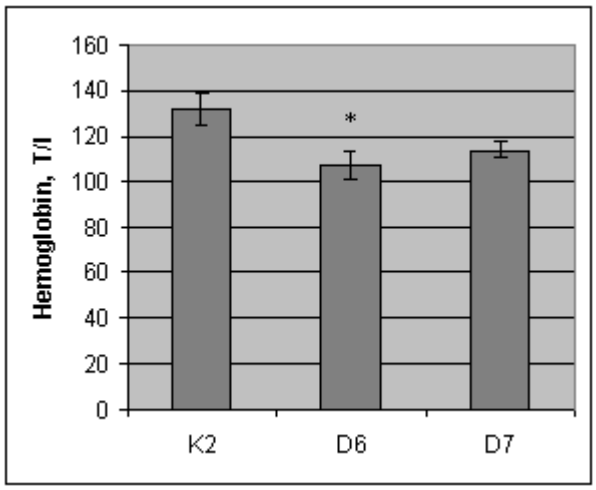

Fig. 3. Hemoglobin concentration in the blood of rats by administration of T-2 toxin and aerosol

Introduction of T-2 toxin causes a reduction in the number haematocrit by 17 % compared to control ($p<0.001$). This may be due to decreased red blood cells from collapsing in the bloodstream. According to the researches, aerosil increases hematocrit by 6 % compared with the group D6 ($p<0.001$) and quantitatively closer to the values inherent in the animals of the control group.

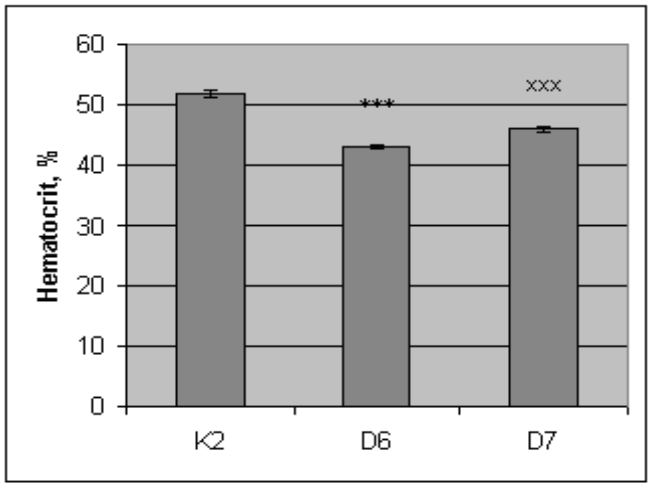

Fig. 4. Rate of hematocrit in the blood of rats by administration of T-2 toxin and aerosil

Changes in mean erythrocyte volume, mean content and high concentration of haemoglobin in the group D6 indicate the morphological and functional changes in the hematopoietic system in rats when toxin is administered. The use of silica has normalized data rates. This indicates a high sorption capacity of aerosil on T-2 toxin in the gastrointestinal tract.

Table 2

Mean corpuscular volume, mean corpuscular Hgb, mean corpuscular Hgb concentration by administration of T-2 toxin and aerosol

	MCV	MCH	MCHC
K2	87.32	22.2	3.93
D6	98.92	24.55	4.03
D7	92.01	23.19	3.96

Received data shows that a single intragastric administration of T-2 toxin to rats causes a reduction in resistance of erythrocytes to the hemolytic, that is explained by activation of free radical oxidation and lipid peroxidation process. With the simultaneous administration of toxin and silica acid resistance of erythrocytes has increased, which can be explained by a decrease adverse effects of T-2 toxin in the body of rats.

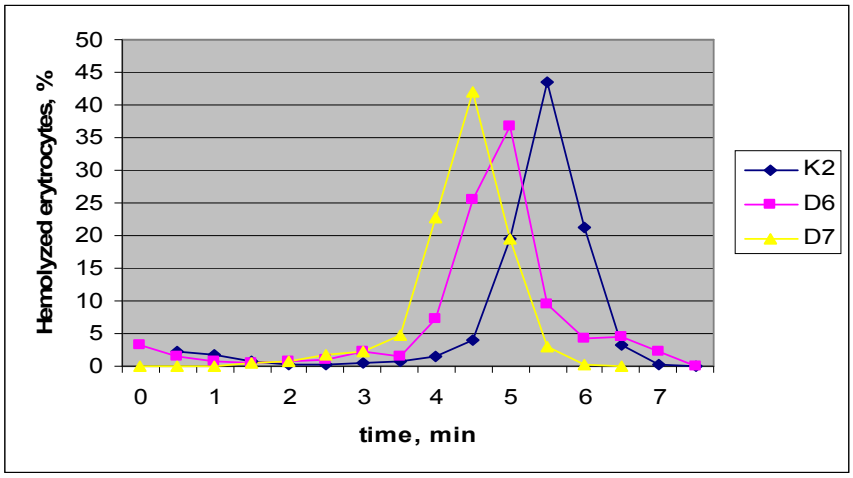

Fig. 5. Acid resistance of erytrocytes of rat blood by administration of T-2 toxin and aerosil

The age distribution of erythrocytes was studied also. During the experiment it was established that the introduction of T-2 toxin causes structural changes in these cells and redistribution of erythrocytes age fractions. Accordingly, on day 4 of the experiment there was a reliable increase ($p<0.1$–$p<0.001$) in population of old red blood cells of D6 by 17 % compared to control K2, which indicates the negative impact of toxin on the structure of membranes. Percentage redistribution of «mature» red blood cells also showed a decrease in their level by 4 %, while the number of «young» erythrocytes not experienced significant changes comparing with control (K2). Introduction of silica resulted in the reduction of the «old» erythrocytes by 8 % compare with the experimental group D6 ($p<0.1$). There is a reduced number of «mature» red blood cells while the number of «young» cells increased by 11 %, which defines the escalation of erythropoiesis by day 4 after toxin administration.

Alterations to the dynamics of reticulocytes exit into the bloodstream indicates the negative impact of T-2 toxin on the hematopoiesis process and erythropoiesis in particular. However, with the introduction of silica to animals of D7 group activation of haematopoiesis process took place.

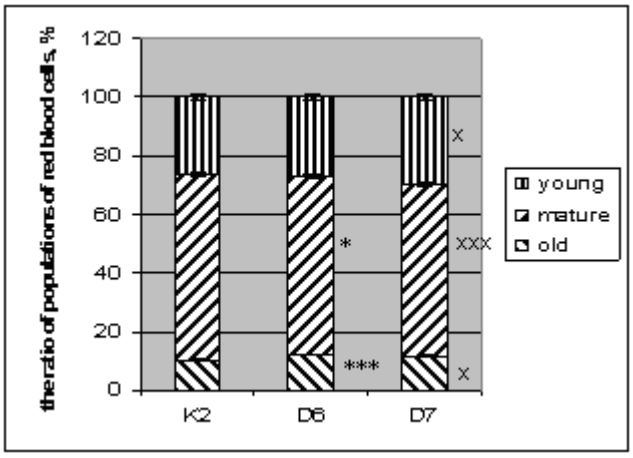

Fig. 6. The ratio of populations of red blood cells by administration of T-2 toxin and aerosil

Received results allow to suggest about high sorption properties of silica in relation to T-2 toxin. The change of haematological parameters and dynamics of reticulocytes exit into the bloodstream indicate the possibility of using silica as a feed additive at the rate of 1 g/kg of feed in order to prevent changes in haematopoiesis process and other negative effects typical with animal mycotoxicoses.

Conclusions

The experimental data showed that a single intragastric administration of T-2 toxin at the dose 1/10 LD50 resulting in credible changes in some haematological indicators, reveal a malfunction of erythron.

So, the results of research pointing at the negative impact of T-2 toxin on the system of erythropoiesis which is manifested in changes of haematological parameters. Reduction in the number of red blood cells and haemoglobin concentration in the animal blood under the influence of the tested toxicant reduces the oxygen supply to the tissues. It is likely that the number of red blood cells decreases due to interruption in the processes of erythropoiesis in the marrow and the growth rate of destruction of red blood cells in the spleen. In general, this impact of T-2 toxin indicate suppression of erythropoiesis and accelerated destruction of erythron cells.

The use of silica has normalized data rates. This indicates a high sorption capacity

of aerosil on T-2 toxin in the gastrointestinal tract.

Prospects for further research. Researches presented in this paper showed a significant effect of T-2 toxin on the erythron system. In the following works we plan to determine the effect of T-2 toxin on other systems and organs.

Next research will be study the changes in the liver and kidneys under the influence of toxin

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