

"MONOSPORIN PK" AND "BACELL" EFFICACY IN TREATMENT OF POULTRY MYCOTOXICOSES

T-2 and HT-2 toxicoses in poultry are caused by chronic exposure to trichothecene mycotoxins contained in the low-quality forage. According to analyses conducted by Mycotoxicology and Mycology Laboratory of the UAAS Institute of Poultry, about 20% of grain and animal feed are contaminated with T-2 and HT-2 toxins [Trufanov O.V., 2005, Kotik A.N. et al., 2006]. Signs of T-2 toxicosis include necrotizing stomatitis, decreased body weight gain, mortality increase, reduction of meat and egg production [Wyatt R. D. et al., 1975], inhibition of protein biosynthesis [Middlbrook J.L. et al., 1989], immunosuppression [Taylor M.J. et al., 1989].

The most effective means of prevention is mycotoxins pollution control at all stages of grain production and storage [Kotik A.M. et al., 2007]. If, however, forage was contaminated and there is no avoidance of its use as feed for poultry, it is necessary to use products reducing the level of the mycotoxins harmful effects. They include adsorbents [Avantaggiato G., 2005] and probiotics [Paškevičius A., 2006]. Effect of sorbents is based on the binding toxins in the intestinal tract and their removal from the body [Dakovic A., 2005]. Although some sorbents effectively bind and hold the individual mycotoxins, all of them have negative side effects:

- 1) sorption of body mineral substances - vitamins, amino acids, polyunsaturated fatty acids, medicines, and beneficial microorganisms;
- 2) mechanical damage to the epithelium of the intestinal mucosa, resulting in penetration of pathogenic microflora;
- 3) high moisture retaining properties.

In view of the above-mentioned shortcomings the use of feed adsorbents should be limited only to those cases where the presence of mycotoxins in feeds is proved. Thus it is necessary to take into account the nature and concentration of mycotoxins, and increase the supply into feeds such substances that may be bound by sorbents.

The action of probiotics is based on the ability of microorganisms to produce enzymes destroying mycotoxins [Trufanov O.V., 2007]. In recent years microorganisms synthesizing carboxyl esterase and epoxide hydrolase have been discovered [Binder E.-M. et al., 2004] transforming trichothecene mycotoxins, lactohydrolase (destroying zearalenone) [Takahashi-Ando N. et al., 2004], fumonisin hydrolases [Duvick J. et al., 1998], UDP-glycosyltransferase that reduce the toxicity of deoxynivalenol [Poppenberger B. et al., 2006] and others. In addition, probiotic micro-organisms produce a variety of biologically active substances that enhance the poultry resistance to the negative effect of mycotoxins. These substances include:

- 1) products suppressing development of pathogenic bacteria - organic acids and natural antibiotics;
- 2) factors increasing bioavailability of nutrients - hydrolytic enzymes and surfactants;
- 3) the essential components of the diet - vitamins and amino acids.

One of the most attractive micro-organisms to generate probiotic products is *Bacillus subtilis*. Bacteria of this type possess of inherent ability to produce all of the above bioactive substances [Priest FG, 1977; Ramos HC, 2000; Stein T., 2005].

"Monosporin PK", based on *B.subtilis* bacterial strain was successfully used for the treatment and prevention of infectious diseases of farm animals, gain increase and improvement of the nutritional value of feed [Boiko N.V. et al., 2006]. In addition, *B.subtilis* bacteria strains being a part of "Monosporin PK" and "Bacell" increase several times the growth rate of cellulose-lytic ruminococci and lactobacilli.

The aim of the research was to study the therapeutic and prophylactic effect of probiotic preparation "Monosporin K" and adding the enzyme probiotic preparation "Bacell" to feed on the course of chicken chronic T-2 and HT-2 toxicoses.

Materials and Methods. Chicken breed "Poltava clay" were used in the research. The chickens were kept in cages under standard conditions (10 heads per section). There were seven groups of 10 chickens (Table 1). Group 1 was control. Chickens of groups 2 – 4 had T-2 toxin (10 mg/kg) added to feed, while HT-2 toxin (16 mg/kg) was supplemented to feed of chickens of groups 5 - 7. Chickens of groups 3, 4, 6 and 7 were given the probiotic product "Monosporin PK" at the rate of 3 ml per 100 heads per day. The feed of groups 4 and 7 included the enzyme probiotic product "Bacell" (2 g/kg feed). All chicken were fed a concentrate feed KK 2-6 for the young poultry (aged 5 - 30 days), according to recommended standards, water was given *ad libitum* after "Monosporin PK".

Table 1

Experimental design

Group	T-2 toxin	HT-2 toxin	«Monosporin PK»	«Monosporin PK» + «Bacell»
1	–	–	–	–
2	+	–	–	–
3	+	–	+	–
4	+	–	+	+
5	–	+	–	–
6	–	+	+	–
7	–	+	+	+

"Monosporin PK" and "Bacell" were kindly provided by Karganyan A.K. ("NIVA" LLC).

T-2 toxin were obtained by adsorption column chromatography by the method previously described from the extract of culture on corn strain *Fusarium sporotrichioides* 2m-15-206 [Kotick A.N. et al, 1979]. HT-2 toxin was obtained by alkaline hydrolysis of T-2 toxin. HT-2 toxin was extracted from the reaction mixture with chloroform and purified by column chromatography distribution.

Test was started with the day-old chickens and carried out for 31 days. Chickens were weighed on the 1st, 8th, 16th, 24th and 31st days. During the test period appearance of signs of necrotic stomatitis were recorded. At the end of the experiment chickens were drugged with diethyl ether, decapitated and blood sampling was performed. The uric acid concentration in blood plasma was determined by reaction with phosphorus-tungsten reagent, creatinine concentration was determined by Jaffe-Popper method and the total protein was checked by biuretic reaction using "Reagent" kits in accordance with the instructions. Carcasses were opened, their pathological and anatomical changes recorded, photographed and disposed of in the Czech pit. The internal organs were weighed and the relative weight was calculated.

Statistical processing was performed by Scheffe multiple comparisons method and by finding Friedman's rank sum criterion [Lapach S.N. et al, 2001]; groups 1 - 3 were not compared to groups 4 - 6.

Results. The results of this experiment are shown in Figures 1, 2, 3 and in Tables 2 and 3.

Toxic effects of mycotoxins. The following has been observed in chickens that received T-2 toxin: a decrease of body weight (Fig. 1), reduction of average daily gain (31%) (Fig. 3), an increase of the relative weight of the heart (16%), pancreas and kidney (from 27% to 52%), reduction in relative weight of spleen (62%) and bursa (31%) (Table 2), 39% reduction of the total protein concentration and 17% decrease of creatinine level in blood plasma (Table 3).

Chickens fed with HT-2 toxin added to feed lagged behind in growth (average daily gain was lower by 14%, Figure 3), showed relatively small live weight, with increased relative weight of kidneys by 1.5 times, low relative weight of spleen (30% less) and elevated levels of uric acid and creatinine in blood plasma (108 and 10%, respectively).

Preventive action "Monosporin PK." As a result of watering chicken with "Monosporin PK" against the background of T-2 toxicosis normalization of body weight was observed – on the 16th and

24th days of the experiment no statistically significant differences with that of the control group were found. The relative weight of heart and kidney was, respectively, 9 and 21% lower compared to the group receiving only the T-2 toxin.

In chickens having "Monosporin PK" added to feed against the background of HT-2 toxicosis the average increase in body weight did not differ from the control. The relative weight of kidneys in chickens treated with HT-2 toxin and "Monosporin PK" was 21% lower, and total protein concentration in plasma is 23% higher than in chickens that received only HT-2 toxin.

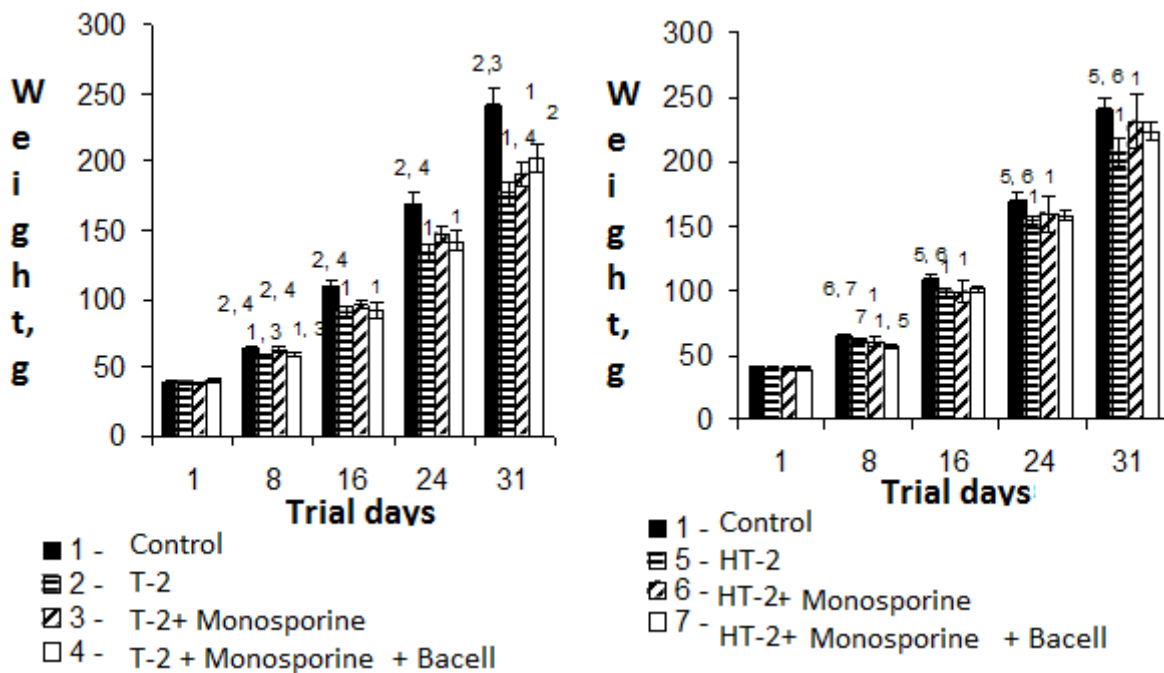


Figure 1. Dynamics of changes in chicken body weight

Note: The figures above the columns indicate the number of groups for which differences were statistically significant (p < 0.05).

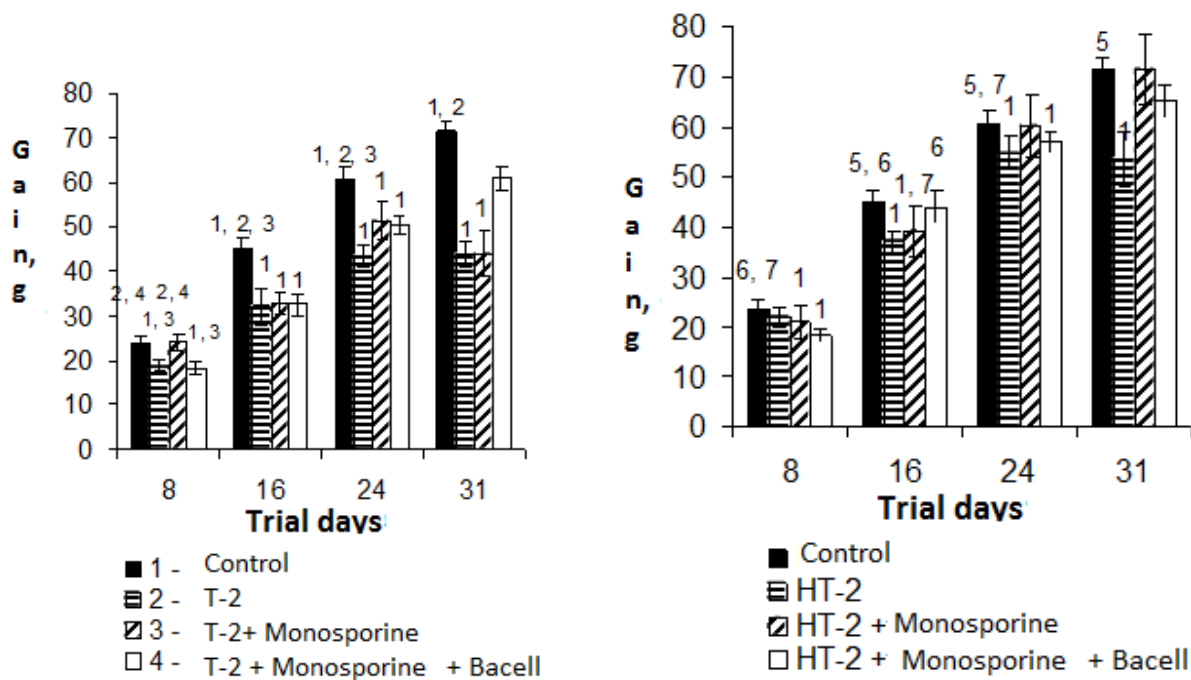


Figure 2. Comparison of average gain of different groups of chickens

Note: The figures above the columns indicate the number of groups for which differences were statistically significant (p < 0.05).

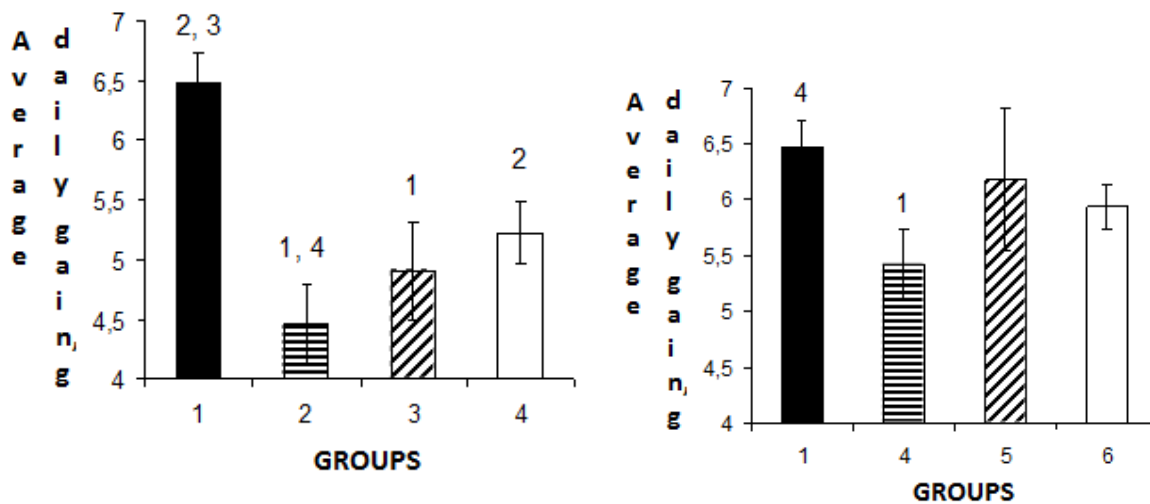


Figure 3. The average daily gain of different groups of chickens

Note: The figures above the columns indicate the number of groups for which differences were statistically significant ($p < 0,05$).

Table 2

The impact of "Monosporin PK" and "Bacell" on the relative weight of chickens internal organs (mg%) with T-2 and HT-2 toxicosis

Group	Heart	Liver	Pancreas	Kidneys	Spleen	Bursa
1	661±16	2935±55 (3)	386±24	930±38	268±27	554±51
2	768±53	3289±180	491±27	1412±111	102±11	382±37
3	698±68	3728±191	502±31	1119±125	122±18	361±47
4	658±24	3012±84	458±27	1204±41	109±15	389±29
5	695±40	3340±230	434±26	1390±62	188±35	482±32
6	693±27	3177±95	431±27	1105±49	178±14	492±34
7	604±13	3330±131	390±17	1212±42	188±15	363±20

Note: The values in brackets indicate the number of groups for which differences were statistically significant ($p < 0,05$).

Table 3

The influence of of "Monosporin PK" and "Bacell" on the diagnostic indices of chicken blood biochemical composition with T-2 and HT-2 toxicosis

Group	Hemoglobin, g/l	Total protein, g/l	Uric acid, mmol/l	Creatinine, mmol/l
1	141±12	40,1±3,7	0,12±0,04	27,4±1,8
2	107±13	28,8±3,5	0,10±0,02	23,4±2,1
3	108±12	27,3±2,7	0,18±0,04	25,5±1,2
4	90±9	28,7±3,1	0,20±0,04	23,3±1,6
5	109±7	34,0±4,9	0,25±0,06	30,0±1,6
6	88±11	44,0±3,8	0,23±0,04	30,0±1,7
7	112±12	32,1±2,4	0,22±0,02	28,4±1,7

Note: The values in brackets indicate the number of groups for which differences were statistically significant ($p < 0,05$).

The effect of simultaneous use of "Monosporin PK" and "Bacell" in case of mycotoxicoses. The use of two products in cases of T-2 toxicosis of chickens led to 13% body weight increase on the 31st day of the experiment compared to the group where only T-2 toxin was used. Live weight of chickens treated with both products in case of HT-2 toxicosis did not differ from the values of this parameter in the control group on the 16th, 24th and 31st days. The average weight gain of groups 4 and 7 for the period from 24th to 31st day of the experiment did not differ from the average weight gain of the control group. Normalization of the relative heart weight and creatinine content in blood plasma should be noted for cases with the combined use of products.

Discussion. As is known, the mechanism of toxic action of trichothecene mycotoxins is inhibition of protein synthesis at all stages of this process [Cundliffe E. et al., 1974, 1977]. One molecule of T-2 toxin or HT-2 toxin is capable of completely "switching off" the entire protein synthesis factory - ribosome

[Middlebrook JL et al., 1989]. The levels of total protein in blood plasma prove this. The toxicity of T-2 and HT-2 toxins in poultry is also reflected in reduced growth rate, deceleration of the internal organs functional activity and suppression of immune system.

One of the reasons for the increase of creatinine and uric acid concentration in blood may be insufficient functional activity of the kidneys, as seen in compensatory increase of their relative weight. It should be noted that the impact of HT-2 toxin negative effect on the kidneys is expressed to a greater extent than that of T-2 toxin. The reason for this difference probably lies in the higher solubility of HT-2 toxin in water, so that it is not deposited in tissues and organs with a relatively high content of lipids, including the liver where the reactions of xenobiotics biotransformation proceed. The unmodified form of HT-2 toxin gets into the kidneys, where it has a toxic effect, and then eliminated from the body by means of filtration.

What is the basis of preventive effect of probiotic products "Monosporin PK" and "Bacell" in cases of T-2 and HT-2 toxicosis? The secret lies in the fact that the bacteria *Bacillus subtilis* being a part of these products possesses a unique family of enzymes - α / β -hydrolases - activated during stress [Brody MS et al., 2001]. These enzymes are characterized by a wide range of catalytic activity, i.e., capable of metabolizing a wide variety of compounds. Stress factors, triggering mechanism for the synthesis of these enzymes may be practically any deviation of normal parameters of habitat *B.subtilis* [Petersohn A. et al., 2001]. For example, in the chicken intestine synthesis of α / β -hydrolases is activated by a relatively high temperature (42°C) and pH fluctuations. Figure 4 shows that these factors trigger the synthesis of carboxylesterase that catalyzes the hydrolysis of the ester groups of T-2 toxin, turning it into a much less toxic metabolite - T-2 tetraol, and epoxyhydrolase, destroying most toxic group - epoxy - resulting in a virtually harmless de-epoxy T-2 tetraol. Firstly this compound is highly hydrophilic, and therefore is not deposited in the tissues and is rapidly excreted from the body, and, secondly, it does not interact with ribosomes, i.e., it does not inhibit protein synthesis [Binder E.-M . et al., 2004]. Thus, carboxylesterase and epoxyhydrolase synthesized by *B. subtilis* perform detoxification of T-2 and HT-2 toxins acting on the various chemical groups of the mycotoxins molecules.

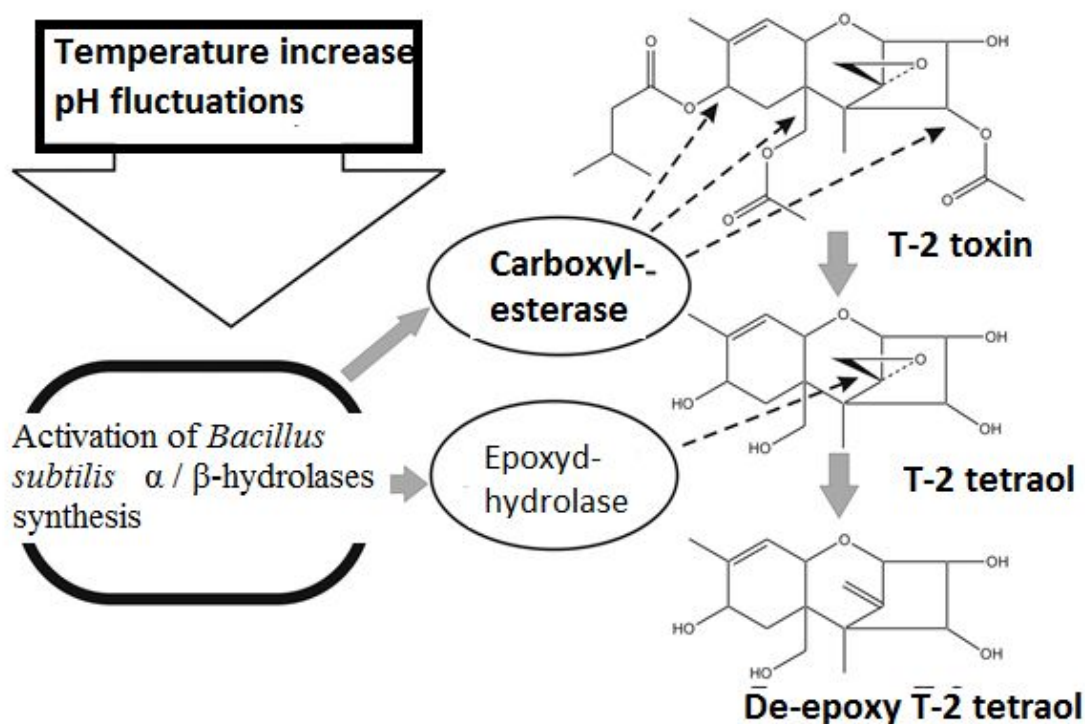


Figure 4. The mechanism of T-2 toxin biotransformation by *B.subtilis* enzymes

Conclusions

1. Consumption of chicken feed containing T-2 toxin at a concentration of 10mg/kg causes a decrease in average daily gain by 31%, increase of the relative weight of the heart, pancreas and kidneys, reduction of the relative weight of the hemopoietic organs - the spleen and bursa, as well as reduction of the concentration of total protein and creatinine in serum.

2. The presence of HT-2 toxin with concentration of 16 mg/kg in chicken feed leads to a decrease in average daily gain by 14%, as well as a negative impact on the kidneys, which is expressed by an increase of their relative weight and build-up of uric acid and creatinine concentrations in blood plasma.
3. The probiotic product "Monosporin PK" has a positive effect on the average daily gain, the relative weight of the heart, kidneys and the concentration of total protein in blood plasma in case of chicken T-2 and HT-2 toxicoses.
4. The combined use of probiotic product "Monosporin PK" and enzyme probiotic product "Bacell" in cases of chicken T-2 and HT-2 toxicoses normalizes such values as live body weight, the heart relative weight and creatinine concentration in blood plasma.