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EVALUATION OF TERRARICH-ANTITOX ON HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS OF BROILERS FED MYCOTOXIN CONTAMINATED RATION AND VACCINATED AGAINST INFECTIOUS BURSAL DISEASE

The search reveal the impact enterosorbent "Terraria ANTITOKS" on hematological and immunological parameters of chickens vaccinated against infectious bursal disease (IBD) on the background of chronic experimental associative mycotoxin. It was found that feeding chickens feed contaminated with fungal toxins (aflatoxin B1, T-2 toxin, deoxynivalenol, zearalenone, ochratoxin, fumonisins) leads to the development of leukopenia in chickens and erythropenia, but these changes in hematological parameters more observed in chickens immunized against IBD on the background of experimental chronic combined mycotoxicosis.feeding chickens enterosorbent "TERRARICH ANTITOKS" normalizes morphological composition of the blood. Immunization of chickens dry live virus vaccine against a strain of IBD "Vinterfild 2512" on the background of chronic combined mycotoxicosis has no significant effect on non-specific immune reactivity and strength of specific post-vaccination immunity.

Key words**:** *enterosorbent, mycotoxins, blood, immune reactivity, chickens, vaccination, infectious Bursal Disease.*

A problem statement

Molds are filamentous fungi that occur in many feedstuffs including grains and forages [8]. Molds can infect animals, especially during stressful periods when they are immune suppressed, causing a disease referred to as a mycosis. Molds also produce mycotoxins, which can cause a mycotoxicosis or toxic response in animals exposed primarily by consuming mycotoxin-contaminated feeds. Surveys reveal sufficiently high occurrences and concentrations of mycotoxins to suggest that they are a constant concern [16].

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The Food and Agriculture Organization [FAO] and other researchers have estimated that worldwide about 25 % of crops are affected annually with mycotoxins [5]. The acute mycotoxicosis outbreaks in modern poultry production system are rare, however, chronic and low level mycotoxin that contaminated grains often causes reduced production efficiency and increases susceptibility to many immune related infectious diseases. Mycotoxins are unavoidable because they are naturally occurring compounds. They contaminate crops before harvest or invade feedstuffs of laying hen during processing, transport or storage [11].

The analysis of recent publications

Many strategies have been tested to avoid mycotoxicosis [7]. Main drawbacks of chemical detoxication are the ineffectiveness against other mycotoxins and the possible deterioration of the animals' health by excessive residual ammonia in the feed. The physical methods are focused on the removal of mycotoxins by different adsorbents added to mycotoxin-contaminated diets [12] with the hope of being effective in the gastro-intestinal tract more in a prophylactic rather than in a therapeutic manner. Certain bacteria, particularly strains of lactic acid bacteria, propionibacteria and bifidobacteria, appear to have the capacity to bind mycotoxins, including aflatoxin and some Fusarium produced mycotoxins [13]. Activated charcoal may be important in binding zearalenone and/or deoxynivalenol [6]. In an *in vitro* gastrointestinal model, activated carbon reduced availability of deoxynivalenol and nivalenol [1].

The addition of mycotoxin binders to contaminated diets has been considered the most promising dietary approach to reduce effects of mycotoxins. The theory is that the binder decontaminates mycotoxins in the feed by binding them strongly enough to prevent toxic interactions with the consuming animal and to prevent mycotoxin absorption across the digestive tract. Therefore, this approach is seen as prevention rather than therapy [2]. Even though food is often contaminated with more than one mycotoxin, most studies are limited to the toxicity of a single mycotoxin.

The aim of our study was to investigate the influence of "TERRARICH-ANTITOX" on the morphology of the hematological and immunological parameters in chickens vaccinated against infectious bursal disease (IBD) on the background of experimental chronic combined mycotoxicosis.

Purpose, objects and methods of research

This experiment was conducted to determine the effect of dietary supplementation of TERRARICH-ANTITOX (lignin derivative, synthesized in Republic of Belarus) on detoxification of mycotoxin in broilers ration and the effect of this adsorbent on blood pictures of broilers. The chicks were reared from 1 to 36 days in the condition of clinic of the epizootiology department, pathanatomy and histology department at Vitebsk State Academy of Veterinary Medicine, Republic of Belarus. A total of 100 chicks, one day old were used. Birds were fed starter diet during the third week of age (beginning

date of experiment; 22,6 % crude protein and 2870,4 kcal/kg of diet) and finisher diet (20,5 % crude protein and 2920 kcal/kg of diet) until the marketing age (36 days of age). Chicks were randomly divided into 5 treated groups, 20 birds for each. First group (G1) fed a ration contaminated with mycotoxin and supplemented with TERRARICH-ANTITOX 5g/kg of diet and vaccinated with IBD vaccine on 15th and 22nd days of age. Second group (G2) was fed a ration contaminated with mycotoxin and vaccinated with IBD vaccine on 15th and 22nd days of age but not supplemented with TERRARICH-ANTITOKS. Third group (G3) was fed a commercial intact broiler ration and vaccinated with IBD vaccine on 15th and 22nd days of age. Fourth group (G4) was only fed a contaminated ration with mycotoxins. Fifth group [G5] was fed a contact clean ration as a control group. The strain of vaccine was interfield 2512 that produced in Russian Federation, the vaccine was supplemented manually intra crop for every chick with one dose. The mycotoxins analyzed in Central Research Laboratory of grain products by ELISA (ridaskrin fast) and the level of mycotoxins were as follows: Aflatoxin B1 0,001 mg/kg, Dezoxivalenol 1,24 mg/kg, Zearalenon 0,068 mg/kg, Ochratoxin 0,005 mg/kg, T2 toxin 0,09 mg/kg, Fuminisen B1 0,2 mg/kg. After 7 days of the first vaccine, 7 days and 14 after second IBD vaccine,Five birds of each group were sacrificed to collect the blood and counting the number of red blood cells, platelets and white blood cells in a counting chamber with the procedure IA Bolotnikov and Y. Solovyov in our modification. Lysozyme activity of blood plasma was measured by procedure of V.G. Dorofeychuku, bactericidal activity – measured by procedure of O.V. Smirnova and GA Kuzmin that modified by Y.M. Markov. The titers of antibodies for infectious bursal disease vaccine were measured by ELISA, using the production system IDEXX laboratories Inc. in Scientific-Research Institute of Applied Veterinary Medicine and Biotechnology, Vitebsk State Academy of Veterinary Medicine.

All data are analyzed by statistical program for study variation statistics, based on the significance (P<0,05). [Microsoft Excel, 2003].

Results and discussion

The effect of mycotoxins was very clear after 7 days of second IBD vaccine in (G4) which recorded the least leukocytes count 22,50±0,56 and the difference was significant from the control (P<0,05), (tab. 1). This results agree with the results of many researchers who reported that T-2 toxin caused leukopenia, and deoxynivalenol caused mild anemia and leukopenia [3, 15]. The effect of mycotoxins in hemoglobin was very clear in (G2) where decrease in hemoglobin (P<0,05) was recorded in comparison with control, this result agree with many scientists who refer that mycotoxins cause anemia and reduction of hemoglobin concentration [4], however, these results disagreed with George et al. (2006) who reported no significant changes in PCV and Hb levels by feeding AF at dietary levels of 50, 150 and 300 ppb in broilers from 0 to 36 days of age [14]. On the other hand, the TERRARICH-

ANTITOX group (G1) is not affected in comparison with the control (P>0,05). The use of mycotoxin binders, or adsorbents, may have the greatest application for routine avoidance of this constant exposure to low levels of multiple mycotoxins. The use of adsorbents to prevent effects of mycotoxins has been actively researched for over 25 years. A number of binder products have been shown effective and their use offers one of the greatest potentials for preventing animal intoxication [10]. There were differences in thrombocytes count and hemoglobin level but these differences were not significant. The effect of mycotoxins with vaccine was very clear after 14 days of second IBD vaccine in (G2) and (G4) which recorded decrease in leukocytes 10^9/L count (P<0,05) in comparison with (G3), but the TERRARICH-ANTITOX group was not affected (P>0,05). The decrease in erythrocytes 10^{12}/L count and hemoglobin level was very clear also in (G2) and (G4) in comparison with the control, but the TERRARICH-ANTITOX group was not affected in comparison with control (P>0,05). There were differences in thrombocytes count but these differences were not significant as shown in.

After seven days of the first IBD vaccine, the differences in ratio of (LASB, %) and (BASB, %) and titers of antibodies against IBD vaccine were very clear but not significant among all groups (tab. 2). The differences between (LASB%) and (BASB) and titers of antibodies against IBD vaccine were very clear but not significant among all groups, and that may be because of big differences in numbers of all groups, but, on the other hand, the titer of antibodies for TERRARICH-ANTITOX group was higher than (G2) with (14,8%) after 7 days after second IBD vaccine and (19,8%) after 14 days after second IBD vaccine. That was a good result that indicated that the TERRARICH-ANTITOX negated the effect of mycotoxins as shown in [9].

Conclusion

Feeding chickens with ration that contaminated with the fungal toxins (aflatoxin B1, T-2 toxin, deoxynivalenol, zearalenone, ochratoxin, fumonisins) leads to the development of leukopenia in chickens and erythropenia. On the other hand, these changes are very pronounced in hematological parameters of chickens that immunized against IBD on the background of experimental chronic combined mycotoxicosis. Supplementing TERRARICH-ANTITOX with a dose 5g/kg of ration essentially negated the effects of mycotoxins on hematological and immunological parameters of broiler chickens. Immunization of chickens dry live virus vaccine against a strain of IBD "Vinterfild 2512" on the background of chronic combined mycotoxicosis has no significant effect on non-specific immune reactivity and strength of specific post-vaccination immunity.

Table 1. **The effect of TERRARICH-ANTITOX in protecting chicken blood parameters**

	Leukocytes,10^9/l	Thrombocytes,10^9/l	Erythrocytes,10^{12}/l	Hemoglobin, g/l
The values after (7) days after first IBD vaccine				
Group 1	24,50±2,25 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	57,00±10,11 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	3,05±0,46 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	75,71 ± 2,26 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05
Group2	23,00±1,69 P_{2-3}<0,05 P_{2-4}>0,05 P_{2-5}>0,05	47,00±11,80 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	2,27±0,05 P_{2-3}<0,05 P_{2-4}<0,05 P_{2-5}<0,05	70,02 ± 3,01 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05
Group3	29,00±1,69 P3-4<0,01 P3-5>0,05	50,50±7,30 P3-4>0,05 P3-5>0,05	3,10±0,33 P3-4>0,05 P3-5>0,05	70,04 ± 8,28 P_{3-4}>0,05 P_{3-5}>0,05
Group 4	21,00±1,12 P4-5>0,05	54,50±14,61 P4-5>0,05	2,86±0,14 P4-5>0,05	74,04 ± 1,13 P_{4-5}>0,05
Group 5	25,50±2,25	59,00±12,92	3,66±0,53	76,06 ± 4,52
The values after 7 days after second IBD vaccine				
Group 1	26,50 ± 1,12 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	58,00 ± 9,55 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	2,85 ± 0,13 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	75,51 ± 5,65 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05
Group 2	24,50 ± 2,25 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	47,00 ± 5,62 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	2,21 ± 0,11 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	68,01 ± 3,01 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}<0,05
Group 3	25,00 ± 3,93 P_{3-4}>0,05 P_{3-5}>0,05	65,00 ± 6,74 P_{3-4}>0,05 P_{3-5}>0,05	2,42 ± 0,11 P_{3-4}>0,05 P_{3-5}>0,05	70,02 ± 1,88 P_{3-4}>0,05 P_{3-5}>0,05
Group 4	22,50 ± 0,56 P_{4-5}<0,05	54,00 ± 6,74 P_{4-5}>0,05	2,23 ± 0,30 P_{4-5}>0,05	69,68 ± 2,26 P_{4-5}>0,05
Group 5	27,00 ± 1,69	46,50 ± 5,62	2,92 ± 0,16	80,74 ± 4,52
The values after 14 days after second IBD vaccine				
Group 1	30,00 ± 1,12 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}<0,05 P_{1-5}>0,05	54,50 ± 14,61 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	2,74 ± 0,13 P_{1-2}<0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	75,38 ± 5,65 P_{1-2}<0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05
Group 2	27,50 ± 1,69 P_{2-3}<0,05 P_{2-4}>0,05 P_{2-5}>0,05	44,00 ± 4,49 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	2,21 ± 0,11 P_{2-3}>0,05 P_{2-4}>0,01 P_{2-5}<0,01	60,97 ± 1,13 P_{2-3}<0,05 P_{2-4}>0,05 P_{2-5}<0,05
Group 3	34,50 ± 1,69 P_{3-4}<0,01 P_{3-5}>0,05	49,50 ± 4,49 P_{3-4}>0,05 P_{3-5}>0,05	2,45 ± 0,27 P_{3-4}>0,05 P_{3-5}>0,05	68,68 ± 0,75 P_{3-4}>0,05 P_{3-5}<0,05
Group 4	26,00 ± 1,12 P_{4-5}>0,05	52,00 ± 6,74 P_{4-5}>0,05	2,37 ± 0,16 P_{4-5}<0,05	66,00 ± 2,63 P_{4-5}<0,05
Group 5	31,00 ± 4,49	62,50 ± 8,99	2,88 ± 0,10	80,40 ± 4,52

Table 2. **The effect of TERRARICH-ANTITOX in protecting chicken immunological parameters**

	LASB,%	BASB,%	Titer of antibodies against IBDV
The values after 7 days after first IBD vaccine			
Group 1	5,25 ± 1,97 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	29,74 ± 6,17 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	198,25±32,02 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05
Group2	6,25 ± 1,40 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	33,64 ± 7,58 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	201,25±40,73 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05
Group3	7,50 ± 0,84 P_{3-4}>0,05 P_{3-5}>0,05	34,27 ± 8,43 P_{3-4}>0,05 P_{3-5}>0,05	183,75±49,74 P_{3-4}>0,05 P_{3-5}>0,05
Group 4	6,75 ± 1,69 P_{4-5}>0,05	28,21 ± 6,55 P_{4-5}>0,05	223,75±80,06 P_{4-5}>0,05
Group 5	6,75 ± 1,40	32,52 ± 8,33	160,00±37,64
The values after 7 days after second IBD vaccine			
Group 1	5,50 ± 0,84 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	33,92 ± 7,43 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	5966,25±1407,31 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}<0,01 P_{1-5}<0,01
Group 2	4,00 ± 0,56 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	31,74 ± 9,96 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	4480,25±1781,19 P_{2-3}>0,05 P_{2-4}<0,05 P_{2-5}<0,05
Group 3	5,25 ± 0,56 P_{3-4}>0,05 P_{3-5}>0,05	28,99 ± 8,55 P_{3-4}>0,05 P_{3-5}>0,05	5580,50±1391,30 P_{3-4}<0,01 P_{3-5}<0,01
Group 4	6,00 ± 1,12 P_{4-5}>0,05	32,73 ± 8,24 P_{4-5}>0,05	234,50±17,17 P_{4-5}>0,05
Group 5	6,75 ± 0,84	30,36 ± 5,90	247,25±78,09
The values after 14 days after second IBD vaccine			
Group 1	7,00 ± 1,12 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	26,91 ± 4,78 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}>0,05 P_{1-5}>0,05	6628,50±558,71 P_{1-2}>0,05 P_{1-3}>0,05 P_{1-4}<0,001 P_{1-5}<0,001
Group2	9,25 ± 2,25 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	29,32 ± 5,83 P_{2-3}>0,05 P_{2-4}>0,05 P_{2-5}>0,05	4646,00±1342,14 P_{2-3}>0,05 P_{2-4}<0,05 P_{2-5}<0,05
Group3	7,75 ± 1,12 P_{3-4}>0,05 P_{3-5}>0,05	23,47 ± 3,18 P_{3-4}>0,05 P_{3-5}>0,05	5613,75±1252,53 P_{3-4}<0,01 P_{3-5}<0,01
Group 4	9,75 ± 1,69 P_{4-5}>0,05	25,37 ± 4,63 P_{4-5}>0,05	188,25±37,64 P_{4-5}>0,05
Group 5	8,75 ± 1,97	29,66 ± 5,52	160,50±45,79

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ПОШИРЕННЯ КИШКОВИХ ПАРАЗИТОЗІВ У СІЛЬСЬКОГОСПОДАРСЬКИХ ПТАХІВ У ГОСПОДАРСТВАХ ЖИТОМИРСЬКОЇ ОБЛАСТІ

В результаті проведених досліджень встановлено, що у центральному районі Житомирщини найбільш поширеними інвазіями кишкового каналу у курей є нематодози (аскаридіоз, гетеракоз) та протозоози (еймеріоз). Інвазії змішані, так як спричинювалися переважно двома видами нематод з найпростішими. Інтенсивність інвазії мала сезонний характер. Аскаридіозно-гетеракозна інвазія склала 57,3 %, а аскаридіозно-гетеракозно-еймеріозна інвазія – 32,7 %. Найвищий показник ЕІ реєстрували восени – 33,0 %, взимку – 28,5 %. Пік еймеріозної інвазії спостерігали навесні, що склала 19,7 %, порівняно з літнім та осіннім періодами.

Ключові слова: *еймеріоз, аскаридіоз, гетеракоз, інвазія, найпростіші.*

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