THE INFLUENCE OF DEOXYNIVALENOL (DON) ON HEMOLEUCOGRAM COMPONENTS AT RATS

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Abstract. Mycotoxins are considered to be natural substances produced by fungi, infecting cereals and other agricultural products. The study was conducted on 20 male Sprague-Dowley rats which received feed with known concentration of DON. The animals were divided in 4 groups: Group I - control group, group II - 3 mg//Kgc DON, group III - 5 mg/Kgc DON, group IV - 9 mg/Kgc DON. The blood samples were taken and the main components of bloods were analyzed: erythrocytes, platelets, leucocytes, Ht. (hematocrit), Hb. (concentration hemoglobin). Variations of blood parameters give information regarding the influence of DON on functionality of cardiovascular and excretory system. Administration of DON contaminated feeds determined at Sprague-Dowley rats a decrease of erythrocytes and platelets with each increasing dose ($p < 0.05$), a decrease of hematocrit and concentration of hemoglobin and no significant changes ($p > 0.05$) were observed regarding the number of leucocytes.

Keywords: mycotoxin, deoxynivalenol, rat, hemoleucogram

INTRODUCTION

Mycotoxins are considered to be natural substances produced by fungi, infecting cereals and other agricultural products. They have a negative effect on human and animal health. They are also thermo stable are easily transmitted by animal products, by technical food processing, from food chains to humans [7].

The production of mycotoxins is not essential for the fungal growth or reproduction, but could be a "virulence factor" for some plant diseases and act against other microorganisms and higher organisms [23]. The degree of fungal growth is influenced by various factors, for example temperature, humidity, rainfall during anthesis (flowering) and at crop harvest, soil treatment and crop rotation [19]. The presence of the toxigenic fungus in a food or feed commodity can indicate a potential hazard, but only the detection of the specific toxin is revealing, since the present fungus is not obliged to produce the toxin; the toxin may persist in the substrate while the fungus could have vanished; the fungus could produce more than one toxin; and one toxin could be produced by different fungus species [12].

Vomitoxine, also known as deoxynivalenol (DON) ((3 α, 7 α, and 15 α trihydroxy 12, 13-epoxytrichothec-9-en-8-one)) is the best known mycotoxin from the trichotecenes group. It is produced by fungi species like *Fusarium, F. graminearum* and *F. culmorum* [16].

There is a different DON dose at every physiological and morphological change. These kinds of changes may take place in humans and animals when DON dose is found in food or feed. Thus, The Scientific Committee on Food [24] derived a tolerable daily intake (TDI) of DON for humans of 1 µg/kg body weight after multiplying a no observed adverse effect level (NOAEL) of 0.1 mg DON/kg LW, resulting from one long-term study in mice, with a safety factor of 100. However, it has to be emphasized that this TDI of DON is not based on its molecular mode of action, but only on growth data of mice. In addition, the orientation value of maximal 1 mg DON/kg in pig diets [5] was also derived primarily from performance of animals.

The deoxynivalenol transfer from feed into human and animal organism is rather easy. There have been many studies to evidence DON concentration from feed and animal organism. It has been established that DON concentration from feed is lower and does not exceed the maximum limit by EU Committee. The DON shall not be found in studied animal blood serum, in case the animal was not eating from the toxic feed [1].

Studies regarding the DON effect were also realized on other groups of organisms like: broiler chickens [4, 15, 25], poultry [3], pig [13], bovine [9] and mice [17].

Observation of total immunity is well elaborated but according to Oswad et al.,[20] it is highly probable that mycotoxins affect predominantly the mucosal lymphoid tissue even before they are absorbed and subsequently metabolized. Presently the use of mycotoxins-binding adsorbents is the most frequently used method of protection of animals against adverse effects of contaminated feed acting directly in the gastrointestinal tract (GIT). The present work has in view to establish deoxynivalenol (DON, vomitoxine) effects on animal health and to bring about new information as concerning the mycotoxines [6].

MATERIALS AND METHODS

In this study we used 20 Sprague-Dowley rats, consulting and respecting European standards for animal rights in laboratory conditions [11].

Animals have been kept in lab, in standard conditions like humidity (45-55%), temperature (25° C) and light control (12h light/ 12h darkness). Each rat has been kept on a normal diet, water *ad libitum* with extra toxic agents. In order to collect their blood, the rats have been anesthetized with ketamine (Calypsol) 30 mg/kg. The rats were separated into four different lots: **Lot I** – formed of 5 rats – the witness lot; **Lot II** – formed of 5 rats treated with 3 mg//Kgc DON; **Lot III** – 5 rats treated with 5 mg/Kgc DON and **Lot IV** – 5 rats treated with 9 mg/Kgc DON. The number of rats on each lot was selected to allow the statistical interpretation of data.

At the beginning of the study, the rats were weighted. Rats from lot number II, III and IV were weighted every week, during two month in order to adjust the toxin doses. DON doses were administered daily, in the form of feeds, during 8 weeks. After 24 h from the last DON administration the rats were sacrificed and blood samples were collected on the anticoagulant for further studies. The blood was collected from inferior cava vein with a syringe and placed in standard blood vacuum containers and anticoagulant substances were added.

The hemoleucogram is represented by: erythrocytes (mil/µl), platelets (mii/µl), leucocytes (mii/µl), Ht (hematocrite) (%), Hb (hemoglobin concentration) (g/dl). In order to establish blood parameters, we used the hemoleucogram analyzer Sysmex SF-3000. Data concerning the studied parameters have been selected and introduced in a computer with the help of Microsoft Excel, a table data base from the software package Microsoft Office 2007 and Minitab, a statistical spreadsheet program.

Results from experimental and control lots have been compared using the Student test (two sample, unpaired, non equal variance) [18]. Interpretation has been made studying obtained p values, with a confidence degree of 0.05 [8].

RESULTS

The rats have been placed in different lots, according to added DON quantity. Blood samples have been taken from every rat. Based on lab studies, we established the following blood parameters: the number of red blood cells, platelets, white blood cells, Ht (%) and Hb (%). For each parameter, determined values have been grouped in tables. Every lot is represented by a value histogram.

As concerning the red blood cells (Fig. 1) obtained values have been represented by average values± standard deviations. Established values are as follows: for Lot I 7.92±0.272, for Lot II 7.358±0.043, for Lot III 7.264±0.028 and for Lot IV 7.136±0.026. It can be observed that with the increasing doses of DON the number of erythrocytes decrease.

Based on histogram values, we have been enabled to determine a comparative study of values. We compared obtained values from the experimental lots where mice have been treated with DON and values obtained from the witness lot. The p value has been established and later compared with p = 0.05, considered a reference value. Thus, p values for Lot I (witness) and Lot II were 0.010.

Statistically, there is a mayor difference between the two lots, as concerning the number of red blood cells. When we compared Lot I (witness) and Lot III it has been established that obtained values is p = 0.006 lower than 0.05 (Fig. 2). There is a mayor difference as concerning the number of erythrocytes. When we compared Lot I (witness) and Lot IV, the established value has been p = 0.003 lower than 0.05. Statistically, there is a substantial difference, as concerning the number of red blood cells (Fig. 3).

Leucocytes (Fig. 4) obtained at studied rats were recorded under an average value± standard deviation. Values are as follows: Lot I 6.30±0.100, Lot II 5.89±0.531, Lot III 6.988±0.703 and Lot IV 5.832±0.420.

The comparative study of experimental variants with the control group showed that there is no significant difference between the control group and each experimental variant (p > 0.05).

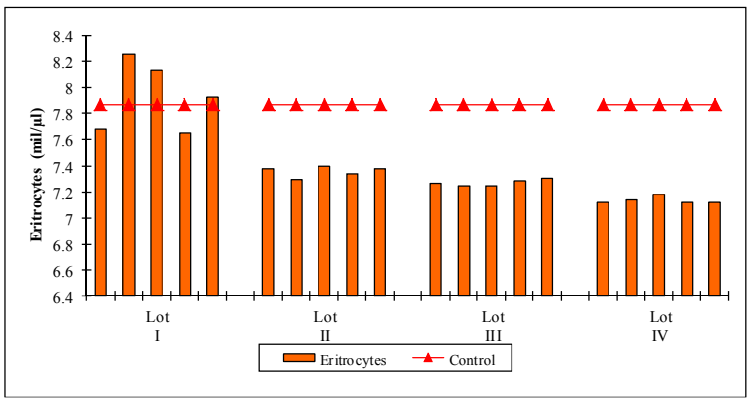

Figure 1. Red blood cell values in experimental lots compared to the control lot

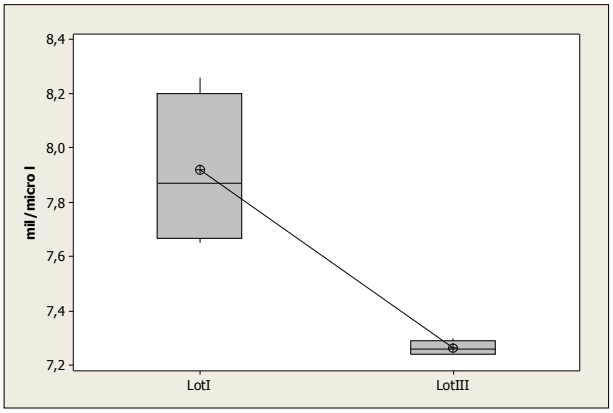

Figure 2. Confidence values between Lot I and Lot III

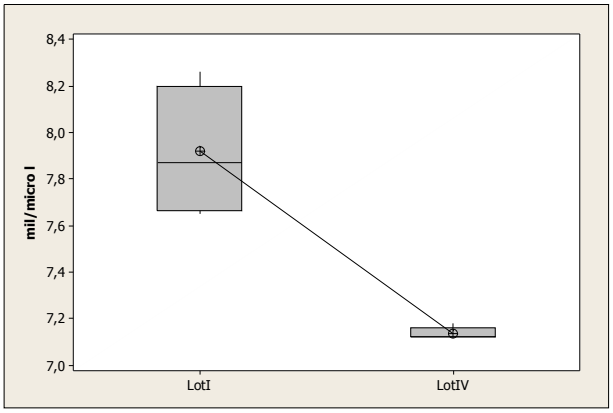

Figure 3. Confidence values between Lot I and Lot IV

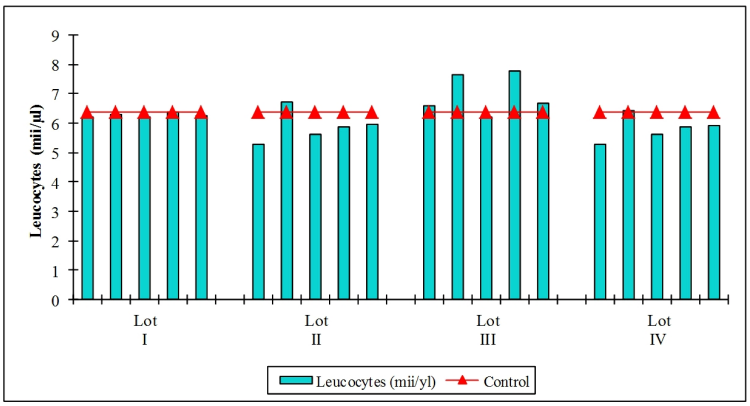

Figure 4. White blood cell values at experimental lots compared to the control lot

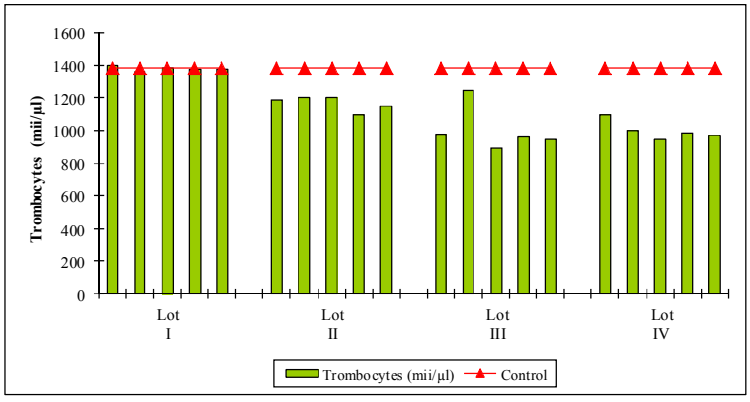

Figure 5. Platelet values in experimental lots compared to the control lot

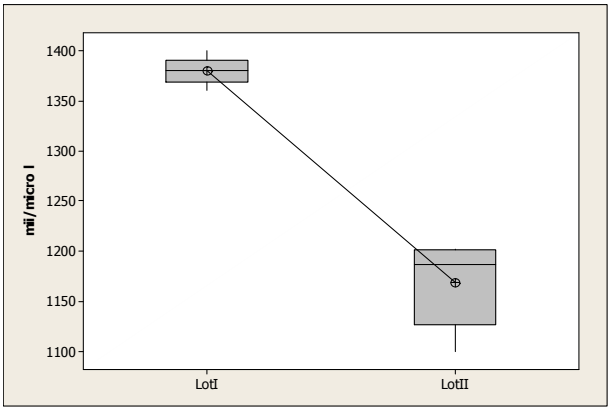

Figure 6. Confidence values between Lot I and Lot II

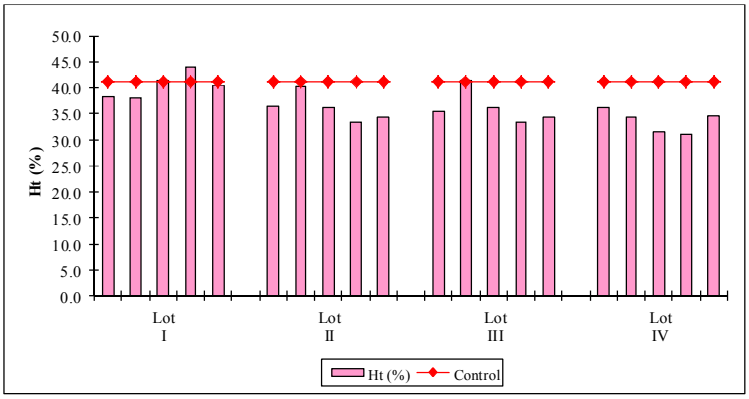

Figure 7. Hemocrotite values at experimental lots compared to the control lot

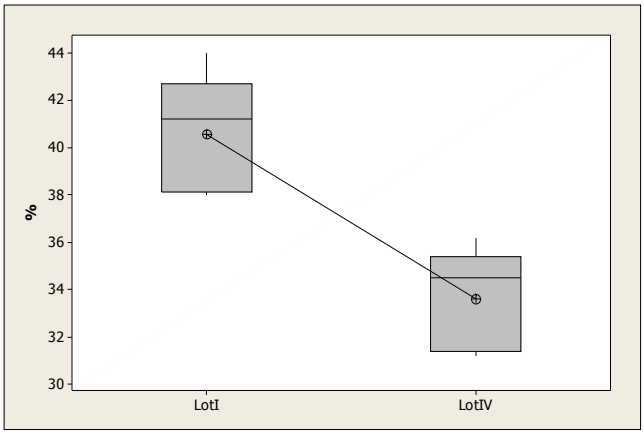

Figure 8. Confidence values between Lot I and Lot IV

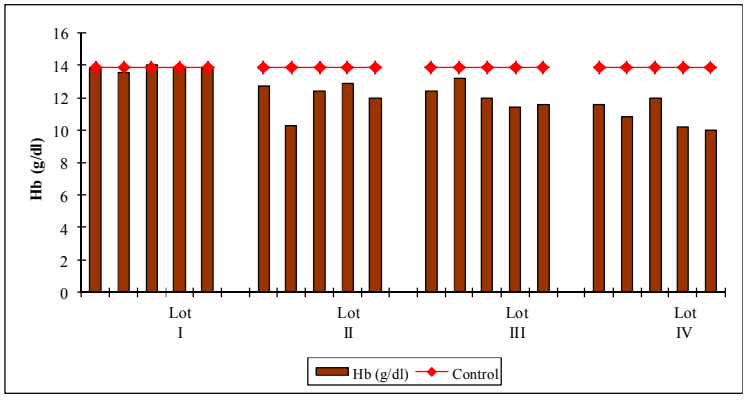

Figure 9. Hemoglobin values at experimental lots compared to the control lot.

The obtained platelet values are showed in figure 5 (Fig. 5). The recorded values are: Lot I 1380±14.12, for Lot II 1169±42.89, for Lot III 1006±139.9 and for Lot IV 1000±59.04.

The obtained values from experimental lots treated with DON and obtained values from the witness lot were compared. In the case of Lot I (witness) and the experimental lot II, p value is 0.00009.

There is an extreme difference between the two lots as concerning the number of platelets (Fig. 6). Comparing Lot I (witness) and Lot III we established the following p values 0.004 lower than 0.05. There is a big difference between the two lots as concerning the number platelets. When we compared Lot I (witness) and Lot IV we established that p values are p = 0.009 lower than 0.05. There is a consequential difference between the two lots, as concerning the number of platelets.

Values established for hematocrites (Fig. 7) are as follows: for Lot I 40.58±2.48, for Lot II 36.14±2.58, for Lot III 36.2±3.09 and Lot IV 33.62±2.14. Comparative study between Lot I (witness) and the experimental Lot II reveals a p value of p = 0.028. Statistically, there are important differences between the two lots, as concerning the hematocrite values. Comparing Lot I (witness) and Lot III, we established that obtained values for p are 0.043 lower than 0.05. There is a significant difference between the two lots as concerning the hemacrotites.

When comparing Lot I (witness) and Lot IV (Fig. 8) we pointed out that obtained values p = 0.002 lower than 0.05. As concerning the two lots, there is a mayor difference between them.

The hemoglobin values were established as follows: for Lot I 13.84±0.151, for Lot II 12.06±1.041, for Lot III 12.12±0.715 and for Lot IV 10.92±0.867 (Fig. 9).

Obtained values in histogram for the hemoglobin concentration have been used to compare studied trust values. Comparing obtained values at experimental lots, mice treated with DON with values obtained from the witness lot. Thus, in the case of Lot I (witness) and the experimental lot II, p values are = 0.019.

There is a significant difference between the two lots, as concerning the hemoglobin concentration. As comparing Lot I (witness) and Lot II, we established p = 0.006 lower 0.05.

There are significant differences between the two lots, as concerning the hemoglobin concentration. Comparing Lot I (witness) and Lot IV, we established p values p =0.002 lower than 0.05. There are important differences between the two lots, as concerning the hemoglobin concentration.

We established that the white blood cells are best represented. The best – known are the neutrophils, monocytes and lymphocytes. Their presence indicates the organism`s tendency to struggle against foreign agents, in our case a chemical agent from feed called DON.

DISCUSSIONS

In our results the DON toxins have a significant effect on erythrocyte synthesis. This shows that the toxin has an inhibition effect in the erythrocyte synthesis or disturbs a link in metabolic activity of erythrocyte synthesis. Other scientific studies shows that the direct effects of DON toxins on tissues and organs are metabolic disturbances like altered nutrient absorption, inhibition of protein synthesis and cytotoxic effects on various cell types [26].

Other authors [2] described a decrease in hematocrit, hemoglobin and erythrocyte counts with a concomitant increase of leukocyte counts after feeding mice 6.25 ppm DON for 91 days. Similar situation is found in our samples except the fact that leukocytes do not show an increase in number at the experimental lots. Leukocytes as the functional cells of the immune system were regarded as a primary target for deoxynivalenol and other trichothecenes [21]. Studies investigating the immunotoxicity of DON, principally in laboratory animals and cell cultures, indicated that DON and other trichothecenes may have stimulatory as well as suppressive effects on immune function and increase the number of leukocytes in blood [17], but in our situation there is no stimulatory effect on leukocytes. Exposure to high doses of trichothecenes resulted in necrosis and atrophy of actively dividing tissues such as bone marrow, lymph nodes, spleen, thymus and intestinal mucosa [2]. In other studies is showed that eosinophil's % activity was increased significantly in DON-administered group of mice as compared to control group [10].

The activity of hematocrit percentage was decreased in experimental lots compared to control. Chinese epidemiological studies suggest that DON may also produce emetic effects in humans [22].

In all experimental lots there is a decrease in number of thrombocytes. Other studies [14] showed that DON toxins have inhibitory effect on thrombocytes activity. Given that critical data gaps still exist regarding the potential health effects of DON, additional research is needed to improve capacity for assessing adverse health effects of this mycotoxin [22].

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