METABOLIC CHANGES IN BITCHES AFTER EXPERIMENTAL ZEARALENONE MYCOTOXICOSIS

MAGDALENA GAJECKA, EWA JAKIMIUK, MAGDALENA POLAK, ŁUKASZ ZIELONKA, KAZIMIERZ OBREMSKI, AND MACIEJ GAJECKI

Division of Veterinary Prevention and Feed Hygiene, Department of Veterinary Health Protection, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
mgaja@uwm.edu.pl

Received for publication September10, 2007

Abstract

The aim of this study was to determine the impact of experimental zearalenone mycotoxicosis on the metabolic changes in the bitches receiving zearalenone for 100 d. The experiment was carried out on 9 sexually mature and clinically healthy bitches. The animals were divided into three groups: DI and DII, which were receiving orally zearalenone at 25 µg/kg b.w. and 50 µg/kg b.w., respectively, and one control group. The haematological and biochemical parameters in blood plasma were measured using laboratory tests. The results of laboratory analyses may suggest that zearalenone apart from the induction of hyperoestrogenism leads to the metabolic dysfunction. After the phytoestrogen was introduced to the blood, it caused anaemia and led to erythropoenia that was accompanied by hypersideraemia and hyperbilirubinaemia. This condition is unfavourable for the excretion of bilirubin with bile acids but it intensifies the decrease in the intensity of hypocholesterolaemia and hyperproteinenaemia. It is assumed that the oral administration of zearalenone in bitches caused changes in the general metabolic profile.

Key words: bitches, zearalenone, metabolic changes.

Material and Methods

All the activities connected with the experiment on animals were carried out in accordance with legal acts binding in Poland that describe conditions and methods for experiments on animals (the opinion of Local Commission of Ethics No. 1/N of 24.01.2003). The experiment was carried out on 9 clinically healthy, 3-year-old bitches of mixed breed, being in anoestrus. Their immunological status had been analysed before the experiment started. The animals were fed standard feed analysed before the feeding for the presence of mycotoxins: aflatoxin, ochratoxin, deoxynivalenole, and zearalenone with negative result.

The bitches were divided into 3 groups: two experimental groups (3 bitches in each), and one control group (3 bitches), according to the suggestions of the European group of toxicologists that perform pharmaceutical tests on dogs as experimental animals (13). The scheme of the experiment was as follows: the bitches of experimental group I (DI) were given zearalenone (ICN Pharmaceuticals Inc.) per os at a dose of 25 µg/kg b.w. for 100 d; the bitches of experimental group II (DII) were given zearalenone per os at a dose of 50 µg /kg b.w. for 100 d; the control group (K) was receiving placebo.

The bitches were divided into 3 groups: two experimental groups (3 bitches in each), and one control group (3 bitches), according to the suggestions of the European group of toxicologists that perform pharmaceutical tests on dogs as experimental animals (13). The scheme of the experiment was as follows: the bitches of experimental group I (DI) were given zearalenone (ICN Pharmaceuticals Inc.) per os at a dose of 25 µg/kg b.w. for 100 d; the bitches of experimental group II (DII) were given zearalenone per os at a dose of 50 µg /kg b.w. for 100 d; the control group (K) was receiving placebo.

The blood samples for biochemical and haematological analyses were taken on the first day from all bitches before the application of zearalenone (group X) and from all groups (DI, DII, and K) on the last day of the experiment. The following biochemical indices were analysed: the concentrations of total cholesterol, urea, sodium, potassium, calcium, inorganic phosphorus, total iron, total protein, oxidative glucose, and total bilirubin, and the activities of alanine aminotransferase (ALT), asparagine aminotransferase (AST), and alkaline phosphatase (ALP). The above parameters were estimated with the use of photometer EPOll-20 with double monochromatisation of radiation.
The picture of metabolic profile in bitches after 100-d of experimental zearalenone mycotoxicosis (\( \bar{x}, \pm SD \))

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>X</th>
<th>DI</th>
<th>DII</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.10±0.24</td>
<td>1.39±0.60</td>
<td>1.40±0.31</td>
<td>2.12±0.38</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.70±0.21</td>
<td>2.66±0.28</td>
<td>3.41±4.33</td>
<td>2.66±0.30</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>6.72±2.18</td>
<td>6.00±2.44</td>
<td>6.62±2.45</td>
<td>6.68±2.21</td>
</tr>
<tr>
<td>Inorganic phosphorus (mmol/L)</td>
<td>2.17±0.74</td>
<td>2.32±1.14</td>
<td>2.08±0.98</td>
<td>2.19±0.88</td>
</tr>
<tr>
<td>Total iron (µmol/L)</td>
<td>24.86±13.29</td>
<td>31.84±21.24</td>
<td>37.91±15.67</td>
<td>25.12±12.86</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>25.12±9.86</td>
<td>27.10±10.49</td>
<td>30.24±22.98</td>
<td>24.07±7.69</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>31.12±9.76</td>
<td>**24.98±10.25</td>
<td>**24.14±9.65</td>
<td>30.00±9.83</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>13.52±2.32</td>
<td>****1.83±0.70</td>
<td>**2.57±2.14</td>
<td>12.24±1.27</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>70.27±6.89</td>
<td>*70.74±7.86</td>
<td>*74.55±7.73</td>
<td>70.76±7.36</td>
</tr>
<tr>
<td>Glucose oxidase (mmol/L)</td>
<td>5.52±1.12</td>
<td>6.04±1.36</td>
<td>**5.17±1.25</td>
<td>5.73±1.47</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>50.12±32.64</td>
<td>**62.98±62.08</td>
<td>*88.01±59.23</td>
<td>52.79±28.72</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>164.83±12.96</td>
<td>163.48±13.01</td>
<td>166.98±13.40</td>
<td>163.05±13.92</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>5.65±1.37</td>
<td>5.49±1.03</td>
<td>5.93±1.50</td>
<td>5.74±1.33</td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td>6.47±0.96</td>
<td>6.36±1.05</td>
<td>6.51±0.91</td>
<td>6.52±0.87</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>73.01±2.71</td>
<td>**68.83±2.49</td>
<td>**70.01±2.83</td>
<td>72.41±2.66</td>
</tr>
<tr>
<td>WBC (10⁹/L)</td>
<td>10.67±2.89</td>
<td>**14.13±3.67</td>
<td>13.90±3.58</td>
<td>10.74±2.54</td>
</tr>
<tr>
<td>Hb (mmol/L)</td>
<td>10.12±0.92</td>
<td>****9.16±1.54</td>
<td>9.69±0.94</td>
<td>10.09±0.90</td>
</tr>
<tr>
<td>Ht (“1”’)</td>
<td>0.47±0.05</td>
<td>**0.44±0.08</td>
<td>0.46±0.06</td>
<td>0.47±0.06</td>
</tr>
<tr>
<td>MCH (fmol)</td>
<td>1.55±0.14</td>
<td>*1.46±0.18</td>
<td>1.51±0.21</td>
<td>1.56±0.15</td>
</tr>
<tr>
<td>MCHC (mmol/L)</td>
<td>21.60±2.04</td>
<td>21.01±2.32</td>
<td>21.58±2.59</td>
<td>21.66±1.98</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Rod neutrophils (“1”’)</td>
<td>0.09±0.05</td>
<td>0.10±0.08</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td></td>
<td>Segmented neutrophils (“1”’)</td>
<td>0.38±0.09</td>
<td>0.39±0.12</td>
<td>0.37±0.10</td>
</tr>
<tr>
<td></td>
<td>Eosinophilic granulocytes (“1”’)</td>
<td>0.01±0.01</td>
<td>0.00±0.01</td>
<td>0.01±0.02</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes (“1”’)</td>
<td>0.42±0.08</td>
<td>0.41±0.13</td>
<td>0.45±0.09</td>
</tr>
<tr>
<td></td>
<td>Monocytes (“1”’)</td>
<td>0.10±0.06</td>
<td>0.10±0.07</td>
<td>*0.08±0.05</td>
</tr>
</tbody>
</table>

*, ** - statistically significant or highly significant differences between the groups DI and DII and the groups X and K
●, ●● – statistically significant or highly significant differences between group DI and group DII

The blood samples for haematological analyses were taken into heparinised tubes and the number of red blood cells (RBC); white blood cells (WBC); mean corpuscular volume (MCV); haemoglobin concentration (Hb); haematocrit (Ht); mean corpuscular haemoglobin (MCH); and mean corpuscular haemoglobin concentration (MCHC) were determined ex tempore. The analyses were performed with the use of haematological analyser HEMOCELL 1600 that determines the number and size of RBC by conductometric method and haemoglobin concentration by colourimetric method. The measurement of the
indices and mean corpuscular volume was controlled by microprocessor. Proportional estimation of the leukocytes was made on fresh blood smears stained according to the May-Grünwald-Giemza method.

With regard to the limit values (norms), the values suggested were used in other studies (1). The statistical analyses were carried out with the use of STATISTICA (data analysis software system) programme, version 6, StatSoft Inc. The analysis of correlation was performed between the groups studied (X, K, DI, DII).

Results

The values of total cholesterol in all experimental groups during the experiment were lower than the physiological range (3.3-9.3 mmol/L) (Table 1). Higher values of calcium were noted in DII group. However, they corresponded to the physiological norms (2.25-3.00 mmol/L). The values of urea were at the upper limit of the physiological norms (3.32-7.47 mmol/L). The values of inorganic phosphorus were at the upper limit of the physiological norms (1.35-2.87 mmol/L). The values of total iron were on the high stable level that was much higher than the physiological norms (16.8-21.8 µmol/L). While assessing the activity of ALP, its increased values were noted in both groups D in comparison with the groups X and K. The values below physiological norms (20-155 IU/L) were noted in both groups X and K. While assessing the activity of ALT in the groups X and K the values obtained clearly differed from the experimental groups, although they were within the norm (3.0-50.0 IU/L). All the values concerning the activity of ALT were definitely lower in both groups D in comparison with the groups X and K. The differences were statistically significant. The values of AST activity in all the groups were very low; however, they were within the physiological norms (1.0-37.0 IU/L). Statistically significant differences were noted between all the groups in the experiment. The values of total protein content were higher than physiological norms (55-70 g/L) in all the groups. Statistically significant difference was noted between the groups DII and DI, and X and K. While assessing the values of glucose oxidase, the statistically significant differences were noted between the group DII and the remaining groups. The values obtained were at the upper limit of the norms (3.9-6.7 mmol/L). The level of total bilirubin was statistically significantly higher (three- or even four-times above the physiological norms – 5.1-20.5 µmol/L) in both groups D and particularly in the group DII in comparison with the groups X and K. The values of sodium exceeded the norms (143.6-156.5 mmol/L) in all the groups. The values of potassium in the groups DII, X and K were above physiological levels (4.1-5.6 mmol/L).

The RBC values were within the physiological norms (5.5-8.0x10^12/L) (Table 1). The WBC values in the groups DI and DII were significantly higher than in the groups X and K. The values in the groups X and K were within the physiological norms (9.0-10.5 x10^9/L).

The concentration of Hb was within physiological norms (7.45-11.17 mmol/L). Statistically significant differences between the groups DI, DII and the groups X and K were noted. The values of Hb were lower in both groups D in comparison with the group X and K. The values of Ht were within the physiological norms (0.37-0.55 1/L). Statistical highly significant differences were noted between the groups D and DII and the groups X and K. The MCV values were within the physiological norms (60-77 fl); however, statistical highly significant differences were noted between both groups D and between the group DI and the groups X and K.

The MCH values in the groups X, K and DII were slightly higher than the physiological norms (1.178-1.488 fmol). Significantly lower values were obtained in the group DI in comparison with the group DII and the groups X and K.

The MCHC values in all the groups were within the lower limits of the norms (19.84-22.32 mmol/L).

The values of red neutrophils were higher than physiological norms (0.00-0.03 "1"). The values of eosinophils obtained were below physiological norms in all the groups (0.60-0.77 "1"). The values of segmented neutrophils obtained were below physiological norms in all the groups (0.02-0.10 "1"). Due to the lack of:>0 value, statistical analyses were not performed on basophils. It is in accordance with physiological norms (0.00 "1"). The values of lymphocytes were higher than physiological norms (0.12-0.30 "1") in all the groups. The values of monocytes were on the upper level of the norms (0.03-0.10 "1") and statistically significant difference was noted between the group DII and the groups X and K.

Discussion

Permanent processes of metabolism and energy have impact on the blood composition. The changes observed in physiological conditions are insignificant. However, each time the regular function of the organs and tissues is disturbed, the imbalance and changes in the blood composition occur. The results of biochemical analyses of the blood serum suggest that microcytic anaemia and leucocytosis mentioned above were accompanied by hypocholesterolaemia, hyperproteinaemia, hypersideraemia, and hyperbilirubinaemia in both experimental groups. Hyperbilirubinaemia is noted in plasma usually as the result of haeme catabolism that leads to an increase in its production or it is as the effect of deficiency in bilirubin excretion. This condition is often noted in different dysfunction of the bone marrow (10) or liver inflammation (6, 14). The increased value of endogenous iron (hypersideraemia) is also noted when the red blood cells haemolysed excessively. The iron of haeme is then included into the total iron in the organism and mainly reused, e.g.: for the production of erythrocytes. When synthesis of erythrocytes is
possible, bilirubin occurs as the result of catabolic processes of haeme.

The concentration of cholesterol in the blood plasma depends on many metabolic processes that are conditioned by genetic factors, nutrition, functions of excretory internal organs, and functional integrity of life vital organs, such as the liver or kidneys (16). The decrease in the concentration of cholesterol in the blood plasma, which was noted during this study, is observed in hepatitis of different degree of severity and under the influence of some different phytoestrogens (5, 7, 12).

The activity of the cellular enzymes – aminotransferases (ALT and AST) that are good indicators of the metabolic activity of the liver, was the following factor analysed in the blood plasma. This study showed significant decrease in their activity in the course of time regarding control group and physiological norms. This indirectly proves the slowdown of the metabolic processes in bitches examined since the activity of these enzymes in the blood plasma increases only when the metabolism accelerates or when the tissues atrophy occurs. While the activity of alkaline phosphatase was analysed, its very low values, as compared to the physiological norms, were noted in the animals examined. However, these values were twice higher in the experimental groups. Hence it can be assumed that increased activity was caused by the factor that evoked inflammation in the liver and led to the increased activity and share of the enzyme (8).

These conclusions are confirmed by the level of total protein that was very high in the experimental animals. It was significantly increased in the group DII. It is dangerous and may condition the occurrence of different bacterial complications. Additionally, hyperproteinaemia and decreased activity of the cellular liver enzymes confirm, at least indirectly, the condition of the hyperactivity of the liver in the synthesis of proteins or hepatitis or simultaneous occurrence of these two conditions (6, 9).

While assessing the erythrocytic values used mainly for diagnosing anaemia (11), the numbers of MCV, MCH, and MCHC were regularly lower in both experimental groups in comparison with the control group and they were within lower physiological norms. This may indirectly prove that probably microcytic anaemia (evoked by iron deficiency) occurs (11).

This condition was accompanied by an increase in the number of leukocytes above the physiological norms. The leukocytes that consist of granulocytes and agranulocytes cooperate by the production of different active substances that protect organism from neoplasms, infections, and toxic agents (e.g. zearalenone) that are deactivated in the liver. Bearing this in mind, it can be suspected that increased number of leukocytes is noted during generalised or systemic inflammation, which is highly probable when the course of metabolic processes are intensified (16).

The results of laboratory analyses may suggest that zearalenone apart from induction of hyperoestrogenism leads to dysfunction of the metabolism, which provokes the cascade of different processes. This phenomenon can be described as follows: initially, after the phytoestrogen is introduced to the blood it causes anaemia and leads to erythroplasia that is accompanied by hypersideraemia and hyperbilirubinaemia as the consequence of disintegration. Simultaneously, provoked dysfunction of the liver occurs. This condition is unfavourable for the excretion of bilirubin with the bile acids but it intensifies the decrease in the activity, hypcholesterolaemia (significant decrease of the concentration of oestrogen precursor), and hyperproteinaemia. In conclusion, the threshold doses of zearalenone applied to the bitches caused changes in general metabolism similar to those reported in zearalenone mycotoxicoses in other species of monogastric animals.

**Acknowledgments:** Financial support was provided by the State Committee for Scientific Research (Project No. PB KBN 2 PO6K 00728) of the Ministry of Scientific Research and Information Technology of Poland.

**References**


progesterone and vitamin E. Mutat Res-Gen Tox En 2005, 565, 139-149.


