INFLUENCE OF LONG-TERM ZEARALENONE INTOXICATION ON THE CONCENTRATION OF PROGESTERONE AND 17β-OESTRADIOL IN BLOOD PLASMA IN BITCHES

MAGDALENA GAJĘCKA, TOMASZ JANOWSKI1, EWA JAKIMIUK, ŁUKASZ ZIELONKA, MAŁGORZATA PODHALICZ-DZIEGIELEWSKA1, KAZIMIERZ OBREMSKI, AND MACIEJ GAJĘCKI

Division of Veterinary Prevention and Feed Hygiene, Department of Veterinary Health Protection, 
1Division of Animal Reproduction, Department of Clinical Sciences, 
Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland 
mgaja@uwm.edu.pl

Received for publication February 14, 2008

Abstract

The experiment was carried out on nine sexually mature, aged 1-3 years, clinically healthy bitches being in anoestrus. The animals were divided into two experimental groups and one control group. The bitches from the experimental groups were receiving zearalenone per os at the doses of 25 µg/kg b.w. and 50 µg/kg b.w., respectively for 100 d. The concentrations of zearalenone, progesterone, and 17β-oestradiol were analysed in weekly intervals. Zearalenone was noted as early as minute 30 and then during the whole experiment. High concentration of zearalenone (9.34 – 124.33 ng/mL) was observed in weeks 6-9. The intoxication was accompanied by hormonal disturbances due to progesterone concentration (to 25-30 ng/mL) depending on zearalenone dose and by the increasing in 17β-oestradiol concentration (to 33 pg/ml). Hormonal disturbances of this kind are similar to those noted in different pathological conditions in the genital tract in bitches.

Key words: bitches, zearalenone, progesterone, 17β-oestradiol.

Zearalenone (ZEA) is non-steroid mycotoxin of oestrogenic properties. It is also a specific hormone that regulates the reproduction of Fusarium (sexual stage - Gibberella zeae). Different kinds of Fusarium produce approximately 150 derivatives of zearalenone under favourable climatic conditions. Hormonal effect of ZEA on the females is the result of its oestrogenic activity (8). It is introduced to the animal organism with the contaminated feed.

In bitches, there are relatively often dysfunctions of the reproduction system, such as: endometritis-pyometra syndrome, prolonged oestrus, ovarian cysts, and others (13). It is supposed that specific for this species hormonal regulation of the reproduction processes, which is based on the long pro-oestrus and oestrus, long progesterone and prolactin cycles, and on high sensitivity to oestrogens, plays an important role in the aetio-pathogenesis of these dysfunctions (7). The application of hormones in bitches for therapeutic and biotechnical (contraception) purposes play an important role (1). There are some suggestions that therapeutic management, unfortunately connected with mistakes in medical art – incorrect application of hormones, is often a cause of pathological condition in reproductive organ in bitches (7). However, another totally unconsidered reason of these disorders may be the fact that bitches take in this mycotoxin with the commercial feed. The research carried out by Zwierzchowski et al., (14) showed the presence of variable concentrations of ZEA in the commercial feed and in some cases these concentrations were estimated as high (14).The significance of this factor is aggravated by the fact that bitches are often fed mono-diet for the period of several months.

The aim of the study was to designate the influence of long-term oral application of zearalenone on the concentration of progesterone, 17β-oestradiol, and zearalenone in blood plasma in bitches.

Material and Methods

All the activities connected with performing the experiment were carried out in accordance with legal acts binding in Poland that describe conditions and methods for experiments on animals. The experiment was carried out on nine bitches (crossbreeds) according to the suggestions of the European group of toxicologists that perform pharmaceutical tests on dogs.
as experimental animals (10). The sexually mature animals aged 1 – 3 years were in anoestrus. They were divided into 3 groups: experimental group I (DI, n=3) receiving 25 µg of zearalenone/kg b.w. (ICN Pharmaceuticals Inc.) every day for 100 d; experimental group II (DII, n=3) receiving 50 µg of zearalenone/kg b.w. in the same manner as group I, and control group (K, n=3) receiving placebo without zearalenone for the same period. Zearalenone and placebo were given per os in the form of the alcohol solution every day before feeding. For the analyses of serum concentrations of zearalenone, progesterone, and 17β-oestradiol, blood samples were collected from each group every seven days at one hour before the morning feeding, for the whole time of the experiment. Moreover, the level of zearalenone, progesterone, and 17β-oestradiol were analysed additionally before and 30, 60, and 120 min after the first application of the mycotoxin in experimental groups. The estimation of zearalenone was carried out according to the common separation techniques with the use of columns of immunological affinity (Zearala-Test™ Zearalenone Testing System, G1012, VICAM, USA) and high performance liquid chromatography (HPLC, Hewlett Packard, type 1050 and 1100) with fluorescent detection. The level of progesterone and 17β-oestradiol was estimated with the use of radioimmunological (RIA) method repeated twice, according to the procedure described by Hoffmann et al. (7). The solutions of progesterone (AS-Gi-P4-IV) and oestradiol (AS-Gi-E2β-1-09/84) antibodies were obtained from Prof. B. Hoffman, University of J. Liebieg, Giessen, Germany.

The results concerning the level of zearalenone were presented with medium values (± standard medium error (SEM)). In order to verify the results statistically, double factor variance analysis (ANOVA) was used. The analyses were processed with the STATISTICA® (Statsoft) computer programme.

Results

Zearalenone concentration (Fig. 1). No ZEA residues were found in the group K. High ZEA levels in blood plasma were noted in both experimental groups in the first two samplings (30-60 min). They were 0.22-5.04 ng/mL at minute 30 and 0.11-4.3 ng/mL at minute 60. The third sampling (120 min after the application of ZEA) did not show ZEA presence in the group DI; however, its concentration in the group DII ranged from 0.16-0.40 ng/mL. In following samplings (days 2-7), the values of ZEA were very low (within 0.2 ng/mL) in the both experimental groups. In week 6 of the experiment the values ranged from 3.00 to 9.34 ng/mL in the group DI and from 4.67 to 124.33 ng/mL in the group DII. The increased levels of ZEA were noted in the groups DI and DII on weeks 7, 8, and 9. Insignificant levels of ZEA were noted on other sampling time-points.

Progesterone concentration (Fig. 2). There were significant discrepancies of data obtained every week; however, they were not revealed by the statistical analysis. The concentration of progesterone of 25 ng/mL and 29.74 ng/mL with decreasing tendency in the following six weeks were noted in the group DI during the first weeks of the experiment. The presence of progesterone in week 5 was demonstrated only in the group DII. Its value reached the highest level (25.21 ng/mL) on week 9. The value decreased since then and it was 4.32 ng/mL on the last week of the experiment. Very low values of progesterone were noted in the group K during the whole experiment (below 1.0 ng/mL).

Fig. 1. Average concentration of ZEA in particular groups of bitches.
Oestradiol concentration (Fig. 3). The groups DI and DII do not differ significantly. The levels of 17β-oestradiol were similar in all the three groups and each sampling time. The level of 17β-oestradiol was higher twice: on week 6 it was ± 21 pg/mL in the group DI, ± 26 pg/mL in the group DII, and ± 25 pg/mL in the group K. The highest levels of 17β-oestradiol were noted on week 13 – DII ± 33 pg/mL and K ± 33 pg/mL. The levels of oestradiol in the first week of the experiment were below 25 pg/mL in all the animals.

Discussion

Oral administration of ZEA to the bitches in anoestrus for 100 d led to a permanent presence of this mycotoxin in peripheral blood. The mycotoxin was detected during the whole experiment. However, the level of ZEA in particular animals and in different stages of the experiment was highly diverse, which was accompanied by different action of the hormones (17β-oestradiol and progesterone). Progesterone occurred in the group DI in early anoestrus (early stage of the experiment) and it was comparable to the result obtained by other authors (6). Progesterone was noted in the second part of the experiment in the group DII; however, its concentration was much higher than in the first part of the experiment. Concentration of 17β-oestradiol showed increasing tendency in both groups with time and its level was 18 to 40 pg/mL, which are very high values (13).

The concentrations of the hormones in the group DI compared to histopathological changes in the uterus described by Gajęcka et al., (3, 4) in bitches, entitle to some suggestions. The occurrence of oedema,
progesterone that resulted in decreasing the level of oestrogens, what was described by Tsumagari et al., (11).

The studies on the long-term experimental ZEA intoxication in bitches in anoestrus suggest that zearalenone mycotoxicosis causes changes in the concentration of 17β-oestradiol and progesterone depending on the dose of ZEA applied to the animals. Lower dose of ZEA caused changes in the concentrations of the hormones analysed, so that the values were similar to those noted in cystic endometrial hyperplasia. ZEA applied in higher doses led to the hormonal condition noted in spontaneous pyometra.

Acknowledgments: Financial support from the State Committee for Scientific Research (Project No. PB KBN 2 P06K 00728) and the Ministry of Scientific Research and Information Technology of Poland.

References


