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# An attempt at inactivation of ochratoxin A in pigs' feed with two feed-added adsorbents

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**Twenty fattened gilts aged about 170 days and weighing 90-100 kg were used in a 14-days feeding experiment (4 groups of 5 animals). Gilts of all groups were offered 3 kg of balanced feed daily. Feed used for control group (C) was free from ochratoxin A (OTA) and from mycotoxins ZEN, AFT, DON, while that for all experimental groups (E1, E2, E3) contained 32,2 µg naturally ocurring OTA/kg. Feed used for group E1 contained an additive of 0.3 kg activated charcoal while that for group E2 – 0.125 kg aluminosilicate/100 kg. Feed used for E3 group contained no OTA-inactivating agents. The post-slaughter laboratory analyses were done of blood serum, kidney, liver and *longissimus dorsi* muscle. All E groups showed similar concentrations of OTA in the examined tissues. Concentration of 32.2 µg OTA/kg feed offered led neither to disease in gilts nor to increase of the creatinine and urea nitrogen level in their blood sera. Both adsorbents did not lead to decrease in micro- and macroelements in the sera of gilts. It is concluded that adsorption of OTA was not effective in its inactivation when adsorbents were used in the applied dozes.**

**KEY WORDS: activated charcoal / adsorbents / aluminosilicate / fatteners mycotoxin / ochratoxin A**

Mycotoxins are secondary metabolites of mould fungi mainly from *Penicilium, Aspergillus* and *Fusarium spp.* which are toxic for the vertebrates. One of them is ochratoxin A (OTA) known as nefropathologic toxin destroying kidney cells. Through the animal products, mycotoxins enter the consumer's organism and promote cancerogenic, nefropathic, neurodegenerative and estrogenic processes [Boyens 2001, Doboszyńska

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*et al*. 2004, Jarczyk *et al*. 2006]. Many research projects attempt at inactivating the feed mycotoxins by adding to the diet substances adsorbing them such as aluminosilicates or activated charcoal [Boyens 2001, Fuchs *et al*. 2002] and the enzymatic substances supposed to decompose mycotoxins. These are yeasts *Saccharomyces cerevisiae* or lactic acid bacteria [Grajewski 2003, Jarczyk *et al*. 1998].

The aim of the present experiment was to estimate the effectiveness of detoxication of OTA present in agricultural crops or in plant ingredients of feeds by adding two adsorbents. Considered was the possibility of inactivation of OTA which accumulates in tissues of fattening gilts as a result of intake of OTA contained in their feed.

## Material and methods

### Animals and feeding

Used were 20 F1 Polish Large White × Polish Landrace gilt fatteners aged about 170 days, of initial live weight 90-100 kg. The animals were randomly divided into four groups of five (control – C, and experimental – E1, E2, E3) and fed, over 14 days, according to the scheme presented in Table 1. The nutritive value of balanced feed for group C (not contaminated with OTA) was similar to that for groups E1, E2 and E3 (naturally contaminated with OTA). The proteinous concentrate was the same in both mixed meals but grain originated from the places of proper *vs*. unproper condition of storing.

**Table 1**. Experimental design – feeding scheme

| Group C – control (5 gilts) | Group E1 (5 gilts) | Group E2 (5 gilts) | Group E3 (5 gilts)* |
|---|---|---|---|
| Nutritionally balanced feed, OTA-free, offered at a rate of 3 kg/gilt/day. No feed refusals; daily ration of balanced feed fully consumed | Nutritionally balanced feed, OTA-contaminated (32.2 μg OTA/kg), offered at a rate of 3 kg/gilt/day (96.6 μg OTA/gilt/day). No feed refusals; ration of balanced feed fully consumed | | |
| | activated charcoal added to balanced feed (0.3 kg/100 kg) | aluminosilicate added to balanced feed (0.125 kg/100)[1] | no additive of any OTA – inactivating agent |

*Two gilts slaughtered 18 hours and three – five hours after the last feeding.
[1]Maximum dose recommended by the manufacturer.
OTA – ochratoxin A.

The powdered activated charcoal CWZ-22 used as adsorbing additive in group E1 was produced by GRYFSKAN, Hajnówka, Poland. According to their laboratory the adsorbing surface of CWZ-22 was 912 $m^2/g$. The aluminosilicate used as additive in group E2 was distributed by FARMWET, Września. According to the manufacturer and distributor the additive was supposed to act as inhibitor of mould biosynthesis of

mycotoxins, to bind them in the digestive tract and to promote the bioneutralization of ochratoxin A in the liver. The additive contains aminosilicates along with herbal and vegetable extracts. According to the GRYFSKAN laboratory, its adsorbing surface was 28 $m^2/g$.

**Analytical**

The feed was analysed for OTA, AFT, DON and ZEN according to instructions of the VICAM Company (Watertown, USA) using Aflatest, Ochratest HPLC, Aflaochra HPLC, DonTest.HPLC and ZearalaTest small columns. The mycotoxin standards used were SIGMA-ALDRICH (Germany). The Shimadzu HPLC was used fitted with UV-Vis, SPD-10 A and Rf-551 detectors. and microbiologically according to Polish Standard [1994]. Both analyses were performed by the Dr. Roman Jędryczko Veterinary Diagnostics Laboratory, 11-036 Gietrzwałd 83, Poland.

After 14 days of experimental feeding all animals were slaughtered (group E3 – 5 or 18 h after the last feeding – Table 1) and blood serum, kidney, liver and l*ongissimus dorsi* muscle tissue were analysed for OTA with the HPLC method as described above.

**Statistical**

The results were evaluated statistically with SPSS programme using one-way Anova.

## Results and discussion

Table 2 presents the OTA content of blood serum, liver, kidneys and muscle of pigs after 14 days of experimental feeding. As expected, OTA was not found in group C. However, all experimental groups showed similar OTA contents of the examined tissues. It proves that additives (activated charcoal or aluminosilicate) were not effective as detoxicants of OTA. It is particularly evident from the comparison of groups E1 and E2 with E3. The lowest concentration was shown in the muscle, the highest in the blood serum and the medium in kidney. The liver had not equalized concentrations of OTA within groups. Five hours since last feeding, the mean OTA content of blood serum (n=3) tended to be higher than in the other E3 gilts (n=2) which were last fed 18 hours before slaughter. The difference was 5.44 µg OTA/L. This indicates that natural removing of the toxin from organisms of adult gilts was possible hence its content of blood serum was relatively low. Hult *et al*. [1980] reported that pig kidney contained 5 times less OTA than blood serum. The results presented in Table 2 show that the OTA content of kidney was only twofold lower.

According to Curtui *et al.* [2001] the highest concentration of OTA in pigs is observed in the blood serum, next in kidney, liver, meat and fat. Similar succession was observed in the present report. In the earlier investigations by Jarczyk *et al*. [1998, 2006] mixed diets for pigs were contaminated with ochratoxin A or zearalenon (32 µg/kg and 500-1100 ppb, respectively). These mycotoxins were not adsorbed by

**Table 2**. Ochratoxin A (μg/kg/L/kg) in the blood serum and tissues of gilts

| Group | | Blood serum | Liver | Kidney | Muscle |
|---|---|---|---|---|---|
| Control (no OTA in feed) | | 0 | 0 | 0 | 0 |
| E1 (OTA + charcoal) | mean | 20.26 | 4.98 | 7.02 | 4.28 |
| | SD | 0.49 | 0.19 | 0.08 | 0.28 |
| E2 (OTA + aluminosilicate) | mean | 17.34 | 9.80 | 8.19 | 3.82 |
| | SD | 0.67 | 1.40 | 0.60 | 0.48 |
| E3 (OTA without detoxicants) | mean | 18.37 | 5.90 | 8.74 | 4.26 |
| | SD | 0.20 | 0.29 | 0.21 | 0.34 |
| In this: 2 gilts fed 18 h before slaughter | mean | 15.65 | 7.38 | 9.15 | 3.20 |
| | SD | 0.43 | 0.42 | 0.34 | 0.01 |
| 3 gilts fed 5 h before slaughter | mean | 21.09 | 4.76 | 8.33 | 5.33 |
| | SD | 0.05 | 0.21 | 0.13 | 0.57 |

**Table 3**. Macro- and microelements in the blood serum of gilts

| Element | Group | Mean | SD | Minimum | Maximum | Reference value |
|---|---|---|---|---|---|---|
| Mg (mmol/l) | C | 1.1 | 0.1 | 1.0 | 1.2 | 0.94-1.46 [Winnicka 2003] |
| | E1 | 1.2 | 0.1 | 1.1 | 1.2 | |
| | E2 | 1.2 | 0.1 | 1.1 | 1.3 | |
| | E3 | 1.1 | 0.1 | 0.9 | 1.2 | |
| Ca (mmol/l) | C | 2.7 | 0.1 | 2.4 | 2.8 | 2.38-3.25 [Winnicka 2003] |
| | E1 | 2.7 | 0.1 | 2.6 | 2.8 | |
| | E2 | 2.7 | 0.1 | 2.5 | 2.8 | |
| | E3 | 2.6 | 0.1 | 2.5 | 2.7 | |
| P inorg. (mmol/l) | C | 3.5 | 0.3 | 3.1 | 3.9 | 1.68-3.10 [Winnicka 2003] |
| | E1 | 3.5 | 0.1 | 3.4 | 3.7 | |
| | E2 | 3.5 | 0.3 | 3.0 | 3.8 | |
| | E3 | 3.4 | 0.3 | 3.1 | 4.0 | |
| Na (mmol/l) | C | 137.6 | 33.2 | 101.0 | 169.0 | 139.1-156.5 [Winnicka 2003] |
| | E1 | 120.6 | 51.0 | 46.0 | 170.0 | |
| | E2 | 167.8 | 28.6 | 144.0 | 214.0 | |
| | E3 | 152.6 | 15.6 | 131.0 | 175.0 | |
| Cu (μmol/l) | C | 27.4 | 3.2 | 24.2 | 32.0 | 24.0- 42.0 [Kuleta *et al*. 1993] |
| | E1 | 27.0 | 2.7 | 24.3 | 31.2 | |
| | E2 | 26.8 | 2.3 | 24.5 | 30.5 | |
| | E3 | 25.1 | 2.5 | 22.0 | 27.0 | |
| Fe (μmol/l) | C | 6.3 | 2.5 | 3.5 | 9.7 | 16.0-25.0 [Kuleta *et al*. 1993] |
| | E1 | 5.2 | 0.6 | 4.4 | 5.9 | |
| | E2 | 8.7 | 5.2 | 4.1 | 17.6 | |
| | E3 | 5.0 | 0.91 | 3.5 | 5.8 | |

aluminosilicates which contained also the *Sacharomyces cerevisiae* yeast enzymes. Significant amounts of both toxins were found in the organs and blood serum of piglets (ZEN) and fattening pigs (OTA).

Discovery of an effective adsorbent for mycotoxins is still an open question. Quite likely, the doses recommended by the producers are too small. Positive results of application of zeolit as feed additive were reported by Boyens [2001] only when its 5% admixture (50 kg/ton) was applied. Adding 1% of zeolit (10 kg/ton) did not affect the amount of zearalenon and α-zearalenon excreted in urine. The high doze of aluminoptiolite in piglets feed (1-2%) reduced OTA in blood serum by about 50% in the experiment in which control group showed 125 µg OTA/L [Fuchs *et al*. 2002]. According to Covart and Casteel [2002] the aluminosilicates have ability of binding the aflatoxins only. However, it is possible that higher doses of detoxicants could be more effective also in the case of other mycotoxins.

Table 3 shows the micro- and macroelements content of blood serum in gilts. In all groups the figures for individual elements are similar, indicating that they were adsorbed neither by activated charcoal nor aluminosilicate.

**Table 4**. Enzyme activity in the blood serum of gilts

| Enzyme (UI/L) | Group | Mean | SD | Minimum | Maximum | Reference value |
|---|---|---|---|---|---|---|
| LDH | C | 1025.2 | 115.2 | 884.0 | 1138.0 | 375-3294 [Winnicka 2003] |
| | E1 | 846.2 | 150.8 | 632.0 | 990.0 | |
| | E2 | 782.8 | 154.2 | 664.0 | 990.0 | |
| | E3 | 797.2 | 218.3 | 609.0 | 1148.0 | |
| ALP | C | 134.4 | 15.2 | 113.9 | 148.0 | 92-294 [Winnicka 2003] |
| | E1 | 97.4 | 13.7 | 82.0 | 119.0 | |
| | E2 | 86.8 | 22.5 | 56.0 | 115.0 | |
| | E3 | 84.0 | 17.9 | 67.0 | 114.0 | |
| CK | C | 10036 | 3174 | 6377 | 14087 | 50-3531 [Winnicka 2003] |
| | E1 | 5010 | 3232 | 1266 | 9746 | |
| | E2 | 4238 | 2036 | 1786 | 6874 | |
| | E3 | 6624 | 3687 | 2967 | 11214 | |
| AspAT | C | 413.0 | 248.9 | 226.0 | 832.0 | 16-65 [Winnicka 2003] |
| | E1 | 360.8 | 234.7 | 105.0 | 618.0 | |
| | E2 | 363.2 | 214.7 | 76.0 | 666.0 | |
| | E3 | 309.6 | 257.7 | 83.0 | 665.0 | |
| AlAT | C | 104.8 | 20.3 | 76.0 | 126.0 | 9-43 [Winnicka 2003] |
| | E1 | 81.6 | 29.5 | 54.0 | 126.0 | |
| | E2 | 72.0 | 21.4 | 46.0 | 94.0 | |
| | E3 | 77.6 | 23.0 | 57.0 | 106.0 | |

Table 4 presents the activity of enzymes in the blood serum. In spite of rather high OTA content of feed, the activities of some enzymes were within, of CK and AlAT were twice higher, and of AspAT – even five times higher than their respective reference values. However, the high enzyme activity concerned all gilt groups, including control animals as well. It indicates that other factor or factors could have affected the high enzyme activity. The animals were in good condition and did not show any manifestation of illness.

Also similar were group means for urea nitrogen and creatinine (Tab. 5). This means that OTA content of feed (32 μg/kg/) was safe for adult gilts when feeding period lasted 14 days only. Jarczyk *et al*. [1998] reported similar OTA content of feed to be related to very high activity of CK enzyme (above 20 000 IU). However, high number of mould CFU/g feed as well as anaerobic bacteria were present in the samples. During this year 42% of corn samples contained more than 5 μg OTA/kg [Jarczyk *et al*. 1998] and 5% contained *Clostridium perfringens* bacteria [Jarczyk *et al*. 1997]. It suggests that bacteria and other contaminating factors could have been the cause of the high activity of some enzymes.

**Table 5**. Concentration of urea and creatinine in the blood serum of gilts

| Item | Group | | | | Reference value |
|---|---|---|---|---|---|
| | C | E1 | E2 | E3 | |
| Urea nitrogen (mmol/l) | 6.20 | 5.64 | 5.30 | 5.90 | 3.32-6.64 [Winnicka 2003 |
| Creatinine (μmol/l) | 144.9 | 159.1 | 156.5 | 177.7 | 88.4-238.7 [Winnicka 2003] |

The results presented here show that adsorption even by activated charcoal, which has extremal adsorbing properties, or by aluminosilicate, are not effective for deactivation of OTA contained in pig feed. The dose of 0.125% aluminosilicate which is recommended by the producer seems to be too low. Both adsorbents used in doses applied in this study did not bind micro- and macroelements in the gilts organism. On the other hand, the content of 32 μg OTA/kg feed fed over 14 days was not shown to affect the pigs health.

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## Próba unieczynnienia ochratoksyny A zawartej w paszy dla świń przez dodawanie do paszy dwóch adsorbentów tej mikotoksyny

S t r e s z c z e n i e

Doświadczenie przeprowadzono na 20 loszkach F1 WBP x PBZ w wieku około 170 dni tuczonych do masy ciała 90-100 kg i podzielonych losowo na cztery grupy po pięć zwierząt. Doświadczenie trwało 14 dni.

W grupie C – kontrolnej – tucznikom zadawano dziennie po 3 kg mieszanki pełnoporcjowej, nie zawierającej ochratoksyny A (OTA) ani mikotoksyn ZEN, AFT i DON.

W każdej z trzech grup doświadczalnych (E1, E2, E3) zadawano tucznikom po 3 kg mieszanki pełnoporcjowej dziennie, zanieczyszczonej w sposób naturalny OTA i zawierającej w 1 kg 32,20 µg tego związku. Znaczy to, że każdemu tucznikowi z grup doświadczalnych zadawano 96,6 µg OTA/dobę. W grupie E1 mieszanka doświadczalna naturalnie skażona OTA, zawierała dodatek aktywowanego węgla drzewnego w ilości 0,3% (0,3 kg/100 kg mieszanki) o powierzchni chłonnej 912 $m^2/g$. W grupie E2 mieszanka doświadczalna zawierała dodatek preparatu glinokrzemianowego o powierzchni chłonnej 28 $m^2/g$ w maksymalnej zalecanej ilości (1,25 kg /tonę paszy). W grupie E3 zwierzęta karmiono mieszanką doświadczalną bez dodatku adsorbentów.

Oznaczano stężenie OTA w surowicy krwi, wątrobie, nerce i w mięśniu najdłuższym grzbietu. Adsorpcja OTA nawet przez aktywowany węgiel drzewny, o olbrzymiej powierzchni chłonnej i przez glinokrzemian okazała się zbyt mała przy zastosowaniu użytej ilości adsorbentów. W badanych tkankach tuczników wszystkich grup doświadczalnych stwierdzono podobne stężenia OTA. Żaden z dwóch adsorbentów nie wpłynął na zmniejszoną koncentrację mikro- i makroelementów w surowicy krwi. Stężenie 32,2 µg OTA/kg paszy nie stało się przyczyną chorób tuczników ani zwiększonej koncentracji kreatyniny i mocznika w surowicy krwi.