THE PRESENCE OF OCHRATOXIN, A RESIDUE IN BLOOD PLASMA OF SLAUGHTERED SWINE

ABSTRACT: The aim and task of this study was to determine the presence of ochratoxin A (OTA) residue in blood of swine, slaughtered regularly. The fact that ochratoxin A is heterogeneously distributed in a contaminated lot of feed material, makes the sampling problematic. It has been shown that an alternative method to monitor the presence of ochratoxin A in the feed is to analyse blood samples from swine, which reflect the toxin content of the ingested feed. With the aim of determining the presence of ochratoxin A residue in blood of swine slaughtered regularly, and originating from different areas of Vojvodina and Serbia, the samples were collected from the corresponding slaughter. During a three month investigation period, a total of 60 blood samples were analysed. Spectrofluorimetric method was applied for sample analysis. The presence of the OTA residue was found in 56.6% of the examined plasma samples. The average OTA concentration in plasma was 2.91 ± 4.91 ng/mL (0.0—33.3 ng/mL). The experiment showed that the average OTA concentration in plasma samples originating from different areas of Vojvodina and Serbia, was not significantly low (p > 0.05).

KEY WORDS: ochratoxin A, plasma, residue, swine

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by several fungi of genera Aspergillus and Penicillium, A. ochraceus principally in tropical parts of the world (23) and P. verrucosum in temperate climates (19). OTA has been found predominantly in cereal and cereal products, but also in a variety of other food commodities (1, 22, 26, 28). OTA has been suggested to be a determinant of a disease known under the name of Balkan Endemic Nephropathy (BEN). This mycotoxin has a special role in the genesis of swine mycotoxic nephropathy (12). Toxin production usually occurs during the storage. The distribute of OTA in the stored grains is very heterogeneous, making analysis and dietary
exposure assessment of animals difficult. However, exposure of animals may also be assessed from analysis of blood levels. The high concentration in a swine blood is due to a relatively long half-life of OTA in this tissue (3.7 ± 6 days) (13), as a consequence of the strong bindings between OTA and serum albumin (7). Hult et al. (1979) demonstrated that the level of OTA in blood is very expressive of the general exposure of the individuals to this mycotoxin, and would be a useful tool in diagnosing ochratoxicosis.

It has also been demonstrated that OTA accumulates in blood and edible organs, especially kidneys. Therefore, pork products, especially those that include blood and kidney, are considered to be an important source of OTA in humans (5). Surveys of swine for OTA in blood and/or edible tissues have been carried out in several countries: Denmark (10), Germany (5, 15, 16, 17), Hungary (25), Norway (15). The purpose of this work was to monitor the presence of OTA in the blood of slaughtered swine, and to investigate its regional distribution.

MATERIALS AND METHODS

Reagents

OTA and Carboxypeptidase A enzyme were purchased from Sigma Chemical Co (St Louis MO, USA). Diluted standard solution of OTA was prepared from a stock solution (10⁻⁴ M OTA) in buffer, which was stored frozen. The concentration of the stock solution was determined spectrophotometrically at 380 nm, using a value of 5680 M⁻¹ cm for an extinction coefficient. Carboxypeptidase A was prepared in 0.04 M tris (hydroxyl-methyl) aminomethane sulfuric acid buffer, pH 7.5, 1 M sodium chloride (100 U/mL). All other solvents and reagents were analytical grade.

Sample collection

Blood serum was collected from slaughtered swine (n=60) originating from three different areas of Vojvodina and Serbia, where a significant swine industry was, during a three month investigation period (April—June). Slaughtered swine were randomly sampled in the slaughterhouse. About 100 mL blood was sampled when slaughtered swine were bled by jugular puncture. Three sodium citrate (TCT) was used as an anticoagulant. Blood samples were centrifuged at 3000 g for 15 min. Serum was decanted and stored at −18°C prior to analysis. Spectrofluorimetric method was applied for sample analysis (9), with detection limit of 2 ng/mL of OTA and 78% recovery.

RESULTS AND DISCUSSION

The results obtained during a three month investigation period are presented in Table 1. The results of this study show that thirty four serum samples
(56.6%), out of 60, were found positive with toxin at levels ranging from 0.0 to 33.3 ng/mL. The average concentration was 2.91 ± 4.91 ng/mL. Of these positive samples, the highest incidence (73.3%), concentration (33.3 ng/mL), and mean level of OTA were found in the samples originating from Kovilj region (5.26 ± 8.22 ng/mL), while the lowest incidence (40%) and mean level of OTA residue were established in the samples originating from Šabac region (1.41 ± 1.86 ng/mL). The results of this study show that the mean level of OTA among the regions where samples were collected, are very similar, but the incidences of OTA are different (Figure 1 and 2). The highest incidence of ochratoxin A residue was established in the samples collected in june (80%), while the lowest incidence of ochratoxin A residue was established in the samples collected in may (20%). During the whole period of investigation, the average OTA differences between the samples originating from the studied regions were not significantly low (p > 0.05).

In comparison with other recently published data about the occurrence and concentration of OTA in blood serum, these results are similar to the studies carried out in other European countries (3, 4, 1, 15, 19, 21). This results indicate that pork products (meat, kidney, blood, and liver) are frequently contaminated with OTA (14, 27). Consequently, foods containing pork liver and blood or plasma, e.g. liver paste and processed meat products, could be important sources of OTA in humans (5, 8). However, when comparing data it should be remembered that factors such as climate conditions during harvest, practices for grain/feed storage, kinds of feed, etc. have influence on the ochratoxin A levels found in swine edible organs. The annual variation and regional differences were primarily due to the moisture content of the grain at the time of harvest. Seasonal variations were also observed after prolonged storage of the grain.

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Positives samples (n)</th>
<th>Positive samples (%)</th>
<th>X ± Sd ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bačka Topola</td>
<td>15</td>
<td>10</td>
<td>66.6</td>
<td>2.17 ± 1.70</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0—5.2)</td>
</tr>
<tr>
<td>Kovilj</td>
<td>15</td>
<td>11</td>
<td>73.3</td>
<td>5.26 ± 8.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0—33.3)</td>
</tr>
<tr>
<td>Šabac</td>
<td>15</td>
<td>6</td>
<td>40</td>
<td>1.41 ± 1.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0—5.0)</td>
</tr>
<tr>
<td>Senta</td>
<td>15</td>
<td>7</td>
<td>46.6</td>
<td>2.66 ± 4.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0—16.0)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>34</td>
<td>56.6</td>
<td>2.91 ± 4.91</td>
</tr>
<tr>
<td></td>
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<td>(0.0—33.3)</td>
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</tbody>
</table>
Fig. 1 — The average concentration of OTA residue during the investigation period

Fig. 2 — The average incidence of OTA residue during the investigation period
CONCLUSIONS

The results of this study show that a thirty four serum samples (56.6%), out of 60, were found contaminated with toxin at levels ranging from 0.0 to 33.3 ng/mL. The average concentration was 2.91 ± 4.91 ng/mL. Of these positive samples, the highest incidence (73.3%), concentration (33.3 ng/mL), and mean level of OTA residue were found in the samples originating from Kovilj region (5.26 ± 8.22 ng/mL), while the lowest incidence (40%) and mean level of OTA residue were established in the samples originating from Šabac region (1.41 ± 1.86 ng/mL). During the whole period of the investigation, the average OTA differences between samples originating from the studied regions were not significantly low (p > 0.05).

The results of this study demonstrate that the detected residue of OTA in blood serum of slaughtered swine, with incidence and mean level of OTA in blood plasma, is comparable to that from the other European countries, but a more extensive survey is advisable in order to obtain a more realistic overview. The distribution of OTA in stored grains is very heterogeneous, alternative method to monitor the presence of ochratoxin A in the feed is to analyse swine blood samples, which reflect the toxin content of the ingested feed. Also, assays of OTA in the blood can provide a level of OTA in other tissues, because blood concentrations are highly correlated with tissue levels. With regard to the national legislation on OTA in animal feed, maximum tolerable levels of OTA are established only for complete feedmixes, intended for swine and poultry, while for feed component they have not been proposed for established yet. In addition, it is necessary to harmonize the national legislation on sampling methods, and OTA limits in animal feed with EU regulations. In order to reduce the colonization of ochratoxigenic mold and toxin production in feed, there is a need for implementation of adequate control of moisture (a_w) and temperature, during transport and storage. Programs such as GAP, GMP, and GHP implemented a HACCP system which is a powerful tool for controlling OTA in commodity system.

LITERATURE


Kotowski, K., et al. (1993): Ochratoxin A residue in kidneys and blood of pigs, Medycyna Weterynaryjna, 49, 554—556
Langseth, W. et al. (1993): Ochratoxin A in plasma of Norwegian swine determined by an HPLC columns witching method, Natural Toxins, 1, 216—221.
Pravilnik o maksimalnim količinama i štetnim materijama i sastojaka u stočnoj hrani (Sl. List SFRJ, 2/90).

ПРИСУСТВО РЕЗИДУА ОХРАТОКСИНА А У КРВНОЙ ПЛАЗМИ ЗАКЛАНИХ СВИЊА

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Резиме

У овом раду приказане су резултати испитивања присуства резидуа охратоксина А у крвној плазми, клинички здравих, редовно закланих, товних свиња. Дистрибуција охратоксина А (OTA) у ускладиштеној храни је веома хетерогена, што доста отежава узорковање, а самим тим и правильну процену алimentaryних изложености људи и животиња овом токсину. Један од альтернативних начина за праћење контаминације храни ОТА је анализи крви која доста познано рефлексује присуство ОТА у храни. За испитивање присуства резидуа ОТА у крвној плазми закланих свиња пореклом из Војводине и дела западне Србије, на линији клана током ветеринарско-санитарног прегледа закланих свиња, методом случајног узорковања узимане су узорци крви свиња. Током тромесечних испитивања укупно је анализирано 60 узорака крвне плазме.

Присуство резидуа ОТА је утврђено у 56,6% испитиваних узорака крвне плазме, док је просечен садржај ОТА у испитиваним узорцима крвне плазме био \(2.91 \pm 4.91 \text{ ng/mL} (0.0—33.3 \text{ ng/mL})\). Нажалост, заступљеност (73.3%), просечен садржај \(5.26 \pm 8.22 \text{ ng/mL}\) и концентрација резидуа ОТА \(33.3 \text{ ng/mL}\) забележени су у узорцима крвне плазме свиња пореклом са локалитета Ковиљ, док су нај-
манаћ заступљеност (40%) и просечан садржај резидуа ОТА (1,41 ± 1,86 ng/mL) забележени у узорцима крвне плазме свиња пореклом са локалитета Шабац. Просечан садржај ОТА у узорцима крвне плазме са одговарајућих локалитета није се статистички значајно разликовao (p > 0,05). Анализа крви доста поуздано рефлектује присуство ОТА у храни и може се успешно користити као средство у дијагностици охратоксиноза, нарочито супклинчих које су најчешће забележене на нашим просторима. На бази познате концентрације ОТА у крви могуће је одредити садржај ОТА у осталим ткивима. Постојећи правилник о максималним количинама прописује максималне количине ОТА само за комплетне смеше и то за свиње и живину, док хранима нису обухваћена. Неопходно је да постојећа за- конска регулатива претрпи знатно веће промене и усклади се са одредбама ЕУ које стандардизују узимање узорака за анализу и садржај ОТА у храни за животине. У циљу спречавања контаминације хране плеснима и ОТА неопходна је примена адекватних мера у контроли температуре и влаге (a_w) током транспорта и складиштења. Применом мера добре пољопривредне прaksi (GAP), добре про- извођачке праксе (GMP), добре хигијенске праксе (GHP), добре складишне прак- се (GSP), имплементираних у систем НАССР представљају ефикасно средство у превенцији контаминације хране плеснима и њиховим токсичним продуктима.