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EVALUATION OF FEED COMPONENTS CONTAMINATION WITH OCHRATOXIN IN VOJVODINA

ABSTRACT: Ochratoxin A is a carcinogenic, teratogenic, immunotoxic and nephrotoxic. The mentioned order stresses the importance of this toxin concerning its harm to human health. The harmful effects of ochratoxin A include the effects at molecular level, such as DNA fragmentation, protein synthesis inhibition, gluconeogenesis, lipid peroxidation, disorder of oxydative phosphorization in mitochondria, inhibition of blood coagulation and apoptosis. The presence of ochratoxin A in a great number of food samples, both of plant and animal origin, is the obvious risk to human health, which is confirmed by the high incidence of this toxin in samples of human serum and milk. It could be stated, with certainty, that the above — mentioned facts are the reason for which the EU has paid great attention to this mycotoxin in recent years.

This paper deals with the results of the analysis of the animal feed component samples for the period 2000—2003 concerning the ochratoxin A content. The analysed feed components were taken from the farms with significant health problems of animals (not monitoring). The samples were analysed by chromatography on a thin layer and with a limited detection method for ochratoxin A of 40 ppb. The analysis was carried out on 108 maize samples, 11 barley samples, 21 wheat samples, 42 sunflower pellets samples and 47 soybean pellets samples (Table 1). The samples of sunflower pellets were contaminated in the greatest percentage, which indicates the inadequate storage of this feed component.

KEY WORDS: barley, maize, ochratoxin A, soybean pellets, sunflower pellets

INTRODUCTION

Ochratoxin A (OTA) is a product of several moulds of the genuses: Aspergillus and Penicillium. In the temperate climate zone this toxin is mostly the product of mould P. verrucosum. A great number of countries, in which P. verrucosum has been indentified as toxin — producing, have registered a significant increase in its production during 2001. At present the toxin — producing P. verrucosum is not only present in Holland, Sweden, Norway, England, Germany and Austria but also in Italy, Spain, France and Portugal. Still P.
verrucosum" is the only ochratoxin producer found. More than 100 isolates of *P. verrucosum* from these environments have been fingerprinted phenotypically and examined for the production of ochratoxin A, and many different clones have been found (Olse n et al., 2002).

According to the other authors *P. verrucosum* sometimes produces, besides ochratoxin A, also citrinin which is considered to be its major synergist (Law i o r, 2001). The most frequent contaminated feed components are maize, wheat, barley and rye. And according to our research a significant contamination of sunflower and soybean pellets has been observed. The infection occurs before harvest and after harvest in storehouses, but the prevalent formation of ochratoxin is during storage. The problem lies in an inadequate drying of feed components prior to storage or the poor storage conditions leading to "hot-spots" of contamination. *P. verrucosum* requires a high water activity (a_w = 0.995), while its other characteristic is the tolerance to high concentration of CO_2 (even 50%).

A good storage practice comprises:
- Design of the top ventilation
- Control of the silo conditions before harvest — to avoid leakage
- Inspection of the upper layer before unloading
- Aeration system in silo
- Grain temperature system

Model describing the risk for condensation of water in the headspace.

In the grinding process, concerning wheat, a part of ochratoxin remains in bran, a part in grinding waste, whereas 50% of ochratoxin A remains in white flour.

In extruded products the quantity of ochratoxin is slightly decreased depending on the temperature, moisture content and screw speed.

Ochratoxin distribution in feed components is rather heterogenic thus making the sampling a critical point in its detection. The sample is taken from different places, its size depending on the amount of the stored food or the size of the means of transportation which makes it possible to avoid taking only contaminated food.

After oral ingestion with foods and feeds, ochratoxin A is slowly absorbed from the upper small intestine. Reaching the systemic circulation, it binds extensively to serum proteins, and translocates to and accumulates in the kidney resulting in measurable residues, whereas lower residual concentrations are found in the liver, muscle and fat. Transfer to milk has been demonstrated in rats, rabbits and humans, but the percentage of ochratoxin A excreted with milk of ruminants is limited, owing to the degradation of ochratoxin A by the rumen microflora (Kwaliteitsreeks nr 89, 2003).

The Scientific Committee on Food expressed the opinion that exposure should be below 5 ng/kg b. w./day (EC, 1998). The Joint Expert Committee on Food Additives, in 2001, retained its previouional Tolerable Weekly Intake of 100 ng/kg b. w. Per week, pending the results of on-going studies on the mechanisms of nephrotoxicity and carcinogenicity (WHO/FAO, 2001). The International Agency for Research on Cancer (IARC) had evaluated ochratoxin A in 1993 (IARC, 1993), and classified it as possibly carcinogenic to humans
(group 2B), based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans.

Approximately 40 countries around the world set regulatory or guideline levels for ochratoxin A in food and animal feed in 2003 (FAO, 2004). In the EU, harmonised regulations exist for ochratoxin A in raw cereal grains including rice and buckwheat, all products derived from cereals, dried vine fruit and baby food (EC, 2002). EU-harmonised specific limits for ochratoxin A in animal feedstuffs have not been proposed yet, but limits have been established at the national level in 8 countries. These countries include Estonia, Lithuania, Slovenia and Sweden, reporting the existence of limits for ochratoxin A in various feedstuffs (including feeds for cattle, pigs, poultry and other farm animals). In addition to legislation on ochratoxin A in animal feed, national legislation exists on ochratoxin A in products of animal origin in three countries: Denmark (for pig kidney), Estonia (for pig liver) and Italy (for pig and derived products) (EFSA, 2004). In our country the maximum quantity of ochratoxin A is regulated by the Act “on maximum quantity of harmful substances and ingredients in animal feed” (1990). The Act “on the amounts of pesticides, metals and metalloids and other poisonous substances, chemotherapeutics, anabolics and other substances which can be found in foodstuffs” (1992) regulates the maximum amount of ochratoxin A allowed in wheat, flour, cereals, coffee, spices, meat and meat products, milk and milk products.

MATERIAL AND METHODS

The analysis of 108 maize samples, 11 barley samples, 21 wheat samples, 42 sunflower pellets samples and 47 soybean pellets samples was carried out. All the analysed feed components were delivered to our laboratory between 2000 and 2003 from the farms with various health problems in animals and from the animal feed plants. The content of ochratoxin in the mentioned feed components was determined by the method according to Balzer et al. (1978). The procedure itself consists of the extraction of ochratoxin A from the sample using acetonitrile, as well as by the extraction and purification of mycotoxin using the chloroform in acid and base media. Qualitative and semi-qualitative determination of ochratoxin A was carried out by chromatography on a thin layer, with a limited detection method for ochratoxin A of 40 ppb.

RESULTS AND DISCUSSION

Ochratoxin A occurrence in animal feed and feed components is predominantly a problem of poor or inadequate drying of cereals prior to storage, or poor storage conditions leading to “hot-spots” of contamination.

The results of our research are shown in Table 1. In the period of three years 229 samples of feed components were analysed, almost half of which were maize samples, which is quite understandable for our region where it is the basic component of a feeding ratio for almost all the animal species, thus
making the maize the most obvious cause of the health problems on a farm. This was not without grounds in this case either as 50% of samples, according to our analyses, were contaminated with ochratoxin A, and 6.5% of the total number of the maize samples were contaminated with the highest amount of ochratoxin of 0.5—1.0 mg/kg. What makes our analyses distinctive compared with the majority of others is a high contamination of sunflower pellets with ochratoxin A both in the previous period (Jurić, 1999) and today. The results of these analyses show a 100% contamination of sunflower pellets sample with 42.8% of the analysed samples of this feed component was contaminated with the maximum amount of ochratoxin A (0.5—1.0 mg/kg).

Table 1. Ochratoxin A content in animal feed components obtained in Vojvodina region from 2000—2003 (mg/kg).

<table>
<thead>
<tr>
<th>Feeds</th>
<th>Total number of samples</th>
<th>Number of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 0.04</td>
</tr>
<tr>
<td>Maize</td>
<td>108</td>
<td>45</td>
</tr>
<tr>
<td>Barley</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Wheat</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Sunflower pellets</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Soybean pellets</td>
<td>47</td>
<td>24</td>
</tr>
</tbody>
</table>

The high prevalence and high levels of OTA contamination in feed grains can be explained by the unfavourable storage conditions, and this finding suggests that OA-related health problems may arise in animals, and that foods of animal origin may be contaminated with this mycotoxin.

According to the EFSA data (2004) there is a significant correlation between ochratoxin A in grains, moisture content, storage period and geographic location. According to the EU data (EC, 2002) for 1500 samples of wheat, oats, rye, barley and maize obtained from 1995—1999 in 11 European countries only 61 samples (4%) contained over 1 µg/kg of ochratoxin A. The other data obtained by EFSA (2004) are 11% of samples with ochratoxin content higher than 1 µg/kg according to the researcher MacDonald or 28% of samples with the ochratoxin A content 1 µg/kg according to Jonsson and Petersson. Particularly high levels of ochratoxin A contamination (1000 µg/kg) were reported in samples of maize, wheat, rye, oats and barley from Austria, Bulgaria, Poland and the Czech Republic. Data from Hungary (Fazekas, 2002) reported results from 30 feeding wheat, 32 feeding maize and 20 feeding barley samples. OTA contamination was found in 26.7% of the feeding wheat, 15.6% of the feeding maize and 35% of the feeding barley samples. The average values and the range of OTA levels found in the above samples were 12.2 and 0.3—62.8 ng/g, 4.9 and 1.9—8.3 ng/g, and 72 and 0.14—212 ng/g, respectively.

Ochratoxin A is lethal in five to six days at 1 mg/kg body weight. OTA fed at 2.5 ppm in the diets of growing pigs was found to reduce growth rates. Levels as low as 0.2 ppm for several weeks can induce detectable renal lesions. Additional clinical signs are diarrhoea, anorexia and dehydration. Someti-
mes clinical signs are not observed, the only gross evidence being the appearance of pale firm kidneys at slaughter (Lawior, 2001). OTA is a carcinogen and nephrotoxin which can enter the food chain resulting in human exposure. As pig herds are exposed to OTA through their feed, their kidneys, liver and pork meat are considered as a possible route of exposure for humans. The programme randomly sampled 300 health and 100 nephropathic pig kidneys in 1997 and 710 healthy pig kidneys in 1998. Less than 10% of samples were significantly contaminated by OTA: in the 1997 survey, 1% of the samples contained 0.40—1.40 μg/kg of OTA and in the 1998 survey 7.6% exhibited OTA levels in the range 0.5—5 μg/kg. In the case of nephropathic kidneys, only traces of OTA (0.16—0.48 μg/kg) were detected in six samples out of 100. Even if not a major route of exposure for humans, pigs are clearly exposed to this mycotoxin and monitoring of pork products and of feed for swine is necessary (Dragacci, 2000). Blood serum, kidney, liver and muscle sample per animal were collected from slaughtered pigs (n = 52) in Romania. A total of 98% serum samples were OTA positive in the range of 0.05—13.4 ng/ml. The incidences of OTA in kidney and liver were very similar (79%, 75%) with mean levels of 0.54 ng/g and 0.16 ng/g, respectively. The lowest incidence (17%) and the lowest mean level contamination (0.15 ng/g) were in muscle samples. The mean distribution in tissues followed the pattern serum kidney liver muscle (100%; 26%, 8.5%; 2.57%) (Curtui, 2001).

Multiple source exposure assessment indicates that the overall contribution of animal products to human exposure does generally not exceed 3—10% (EFSA, 2004).

CONCLUSION

The conclusion of this paper could be the recommendation of EFSA (2004) to all EU member states and future member states.

1. There is a need to establish measures to reduce the formation of ochratoxin A in feed commodities during transport and storage, including on-farm storage, and to implement adequate control of moisture (water activity) and temperature changes during storage.

2. Analytical methods with appropriate limits of quantification for feeding stuff need to be validated by collaborative studies.

3. The efficacy of feed control programmes should be assessed by surveys of blood levels of ochratoxin A in pigs at slaughter.

4. More data are needed in order to establish a NOEL for pigs and poultry.

5. In order to assess the significance of residue levels in animal tissues, both with respect to animal health and to human exposure, more extensive occurrence data on ochratoxin A in animal tissues and products thereof and from other foods, covering all member states, are required.

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LITERATURE


ЕВАЛУАЦИЈА КОНТАМИНАЦИЈЕ ХРАНИВА ЗА ЖИВОТИЊЕ ОХРАТОКСИНОМ А У ВОЈВОДИНИ

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Резиме

Охратоксин А је канцероген, тератоген, имунотоксичан и нефритоксичан. Набројани редослед даје важност овом токсину у погледу штетности људском здрављу. Штетности охратоксина А укључују ефекте на молекуларном нивоу, као што су оштећења ДНА, инхибиција синтезе протеина, глутаонегенеза, липидне пероксидације, помећај оксидативне фосфорилације у митохондријама, инхибиција згрушувања крви и апоптоза. Присуство охратоксина А у великом броју узорака хране, како биљног тако и животињског порекла, евидентан је ризик за људско здравље, што потврђује висока фреквенција присуства овог токсина у узорцима хуманог серума и млека. Могло би се са сигурносћу рећи да су управо напред наведене чињенице разлог што EU последњих неколико година највећу пажњу поклања овом микотоксину.

У овом раду приказани су резултати анализе узорака хранива за животиње за период 2000/03. године на садржај охратоксина А. Анализирања хранива потиснула су са фарми на којима су били израђени здравствени проблеми код животиња (није мониторинг). Узорци су анализирани хроматографијом на танком слоју и са лимитом детекције методе за охратоксин А од 40 ppb. Анализирани је 108 узорака кукуруза, 11 узорака јечма, 21 узорак пшенице, 42 узорка сунцокретове сачме и 47 узорака сојине сачме (Табела 1). Узорци сунцокретове сачме контаминирани су у највећем проценту, што указује на неадекватно складишење овог хранива.