Original paper

PRESENCE OF AFLATOXIN IN COMPLETE FEEDING MIXTURES FOR DIFFERENT CATEGORIES OF PIGS IN SERBIA

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Abstract

A total of 106 samples of complete feeding mixtures for different categories of pigs were analyzed for presence of aflatoxin. Feeding mixtures were composed of feed materials harvested during 2012 harvest season in Serbia. These samples included complete feeding mixtures for fattening pigs (49), lactating sows (19), pregnant sows (10) and piglets (28). Analysis was performed using ELISA test kits. The presence of aflatoxin was the highest in complete feeding mixtures for fattening pigs (43%), somewhat lower in complete feeding mixtures for lactating sows (37%) while in complete feeding mixtures for piglets was 25% of positive samples. Incidences ranged from 4 µg/kg to 66 µg/kg, both in complete feeding mixtures for fattening pigs. Out of 10 samples of complete feeding mixtures for pregnant sows, aflatoxin was detected in only one sample in the concentration of 14 µg/kg. Contamination levels were above the maximum limit established by the European Union and Serbian regulation in 7 (14%) samples of complete feeding mixtures for fattening pigs, 4 (21%) samples of complete feeding mixtures for lactating sows and in 7 (25%) samples of complete feeding mixtures for piglets. None of the samples of complete feeding mixtures for pregnant sows contained aflatoxin above the maximum permitted level.

Key words: *aflatoxin, ELISA, feedstuffs, pigs, Serbia*

Introduction

Mycotoxins, as natural food and feed contaminants, became a public health problem of considerable importance. Fungal invasion and subsequent mycotoxins production can occur during plant growth, maturity, harvesting, storage, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) (Bernardo, 2004; Lanyasunya et al., 2005). The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins (WHO, 1999). Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2), a group of potent carcinogenic and teratogenic compounds, are secondary metabolic products of some *Aspergillus spp.: Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* (Kurtzman et al., 1987; Martins, 1989). Among all aflatoxins, the aflatoxin B1 (AFB1) is the most potent hepatocarcinogenic substance known; recently, after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011).

Aflatoxins may cause various performance and health problems in pigs, including significant effects on growth performance, decreased average daily gain and feed gain ratio, increased relative weights of liver, kidney, spleen and pancreas, impaired liver function, the increase of serum enzyme activities and the decrease of liver enzyme activities (Shi et al., 2007). Aflatoxins have a severe influence on reproduction in sows which may include endometritis, mammary glands edema and reduced rate of piglets' survival (Liu et al., 2009).

All mammals that ingest AFB1 during lactation excrete aflatoxin M1 in their milk (Wood, 1991), even though its amount depends on many factors. In case of dairy cows, the excreted amount of AFM1 is up to 6.2% of ingested AFB1 (Veldman et al., 1992). It was reported that in case of pigs, an AFB1 conversion index from feed to liver is 800:1 and that the carry-over of AFB1 from sow feed to milk was not known (Stoloff, 1977). Crenshaw (2008) reported the presence of AFM1 in the milk of sows fed diets containing aflatoxins. It was found that pigs nursing sows consuming feed with 500 to 750 ppb of aflatoxin had higher death rates and slower growth. Also, even though pigs were not exposed to aflatoxins after weaning, they were permanently stunted and performance was reduced throughout the growing/finishing period. Bertuzzi et al. (2003) investigated the AFB1 carry-over in lactating sows. Starting from parturition, lactating sows were fed 5 kg of contaminated feed for 7 days. Colostrum and milk samples were collected daily for 10 days after parturition, from each sow. The feed was prepared by adding 5% of a peanut meal naturally contaminated by aflatoxins and contained a mean level of 6.4±0.5 and 0.67±0.05 ppb of AFB1 and AFB2, respectively. The authors concluded that the AFB1 carry-over as AFM1 in the milk was much lower in sows compared to cows (1 - 3%).

To reduce the risk of exposure, many countries have regulated the levels of aflatoxin in feed. Currently, the legal limits of aflatoxin in feedstuffs are highly variable from the European Union (EU) countries to other countries. The EU has a limit of 20 μg/kg for complete feedingstuffs for pigs and poultry (except young animals) and 10 μg/kg for other complete feedingstuffs (European Commission, 2003). In Serbia, proposed maximum permissible levels of aflatoxins in complete and complementary feedingstuffs for pigs and poultry (except young animals) is 20 μg/kg and in complete and complementary feedingstuffs for calves, lambs, kids, piglets, chicks, turkeys, ducklings is 5 μg/kg (the Official Gazette of the Republic of Serbia, 27/2014).

Aflatoxin has long been monitored by the United States Food and Drug Administration, and a level of 20 μg/kg has been set as the limit of aflatoxin content for corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals (United States Food and Drug Administration, 2000). The mentioned regulation also establishes maximum level of aflatoxin in cottonseed meal intended for beef, cattle, swine, or poultry (300 μg/kg), corn and peanut products intended for finishing swine of 100 pounds or greater (200 μg/kg) and corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry (100 μg/kg) (United States Food and Drug Administration, 2000).

The purpose of this study is to give the information on the occurrence of aflatoxin in complete feeding mixtures for different categories of pigs.

Materials and methods

Samples- One hundred and six samples of complete feeding mixtures for different categories of pigs were collected randomly from different farms in Serbia during 2013 year. Immediately after sampling, 1000 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. After that, the sample was homogenized by mixing. Samples thus prepared were packed in plastic bags and stored in a freezer at -20 ºC until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction- Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

Analysis- The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities on the basis of which aflatoxin content was calculated, were read using the reader of microtitration plates with a 630 nm filter (BioTec Instruments, USA).

Results and discussion

Validation parameters of method for aflatoxin determination were estimated according to the European Commission (2006) and Reason (2003). Limit of detection (LOD) for aflatoxin in feed samples was 0.45 µg/kg, while limit of quantification (LOQ) was 1.37 µg/kg. Average recovery value, based on analysis of certified reference material, was 106.2% which is within acceptable limits according to the European Commission (2006). Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable". Measure uncertainty was calculated in accordance with Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories (Magnusson et al., 2012) and it was 34.55%. The obtained results showed that the proposed analytical method fits well for control purposes of aflatoxin in feed samples.

Table 1. *Presence of aflatoxin in samples of complete feeding mixtures for different categories of pigs*

Feeding mixture	No of samples	No of positive samples (%)	No of samples above maximum permitted level (%)
Fattening pigs	49	21 (43)	7 (14)
Lactating sows	19	7 (37)	4 (21)
Pregnant sows	10	1 (10)	-
Piglets	28	7 (25)	7 (25)
Total	106	36 (34)	14 (13)

The occurrence of aflatoxin was investigated in 106 samples of complete feeding mixtures for different categories of pigs and the results are presented in Table 1 and Table 2. Out of 106 samples, aflatoxin was present in 36 (34%) samples with the average content of 20 µg/kg, ranged from 4 to 66 µg/kg. The highest rate of positive samples (43%) and the greatest concentration of aflatoxin (66 µg/kg) was established in samples of feeding mixtures for fattening pigs. It needs to be pointed out that this was the largest group of samples (nearly half of samples). Despite the smaller number of samples in case of feeding mixtures for lactating sows, the presence of aflatoxin was quite high (37%) with the highest mean value of 27 µg/kg. Somewhat lower incidence was established in samples of feeding mixtures for piglets (25%) with the average aflatoxin content of 16 µg/kg, while in samples of feeding mixtures for pregnant sows, aflatoxin was found in only one sample.

Regarding legislative in our country and the European Union, contamination levels were above the maximum limit in 7 (14%) samples of complete feeding mixtures for fattening pigs, in 4 (21%) samples of complete feeding mixtures for lactating sows and in 7 (25%) samples of complete feeding mixtures for piglets.

Table 2. *Aflatoxin concentration in samples of complete feeding mixtures for different categories of pigs*

Statistical parameters	Feeding mixture				
	Fattening pigs	**Lactating sows**	**Pregnant sows**	**Piglets**	**Total**
No of samples	49	19	10	28	106
Average (µg/kg)	19	27	14	16	20
Std. dev. (µg/kg)	16	17	-	19	17
Minimum (µg/kg)	4	9	-	6	4
Maximum (µg/kg)	66	59	-	58	66

During 2012 the presence of aflatoxin was found in most grains in the Republic of Serbia, particularly in maize (Kos et al., 2013a; Jajić et al., 2013b) which may explain the obtained results. Jajić et al. (2013b) reported aflatoxin contamination in 63.6% of the analyzed maize samples with concentration range of 5-367 µg/kg. Somewhat lower contamination (56.4%) was reported by Kos et al. (2013a) with a concentration range of 1-80 µg aflatoxin/kg. All this has contributed to the aflatoxin contamination of compound feed (Jajić et al., 2013a). Extreme aflatoxin contamination of crops and feed seems to be a consequence of favorable weather conditions for Aspergillus mold growth and aflatoxin production. Kos et al. (2013b) compared climatic conditions in period 2009 – 2012 and concluded that very high average temperatures and greater number of days with temperatures higher than 30 °C and 35 °C characterized summer of 2012. According to Republic Hydrometeorological Service of Serbia (2012) drought period (July-August) was characterized with extremely high temperatures and lower amount of precipitation for up to 95% than multiannual average. Kos et al. (2013b) noted that different weather conditions during 2012 in comparison to previous years resulted in presence of aflatoxins in maize.

As for our neighboring countries, aflatoxin contamination was monitored in Romania (Braicu et al., 2008; Tabuc et al., 2010) and Croatia (Šegvić Klarić et al., 2009). Tabuc et al. (2010) analyzed 56 cereal samples (corn, wheat, barley and oats). They determinated the level of fungal contamination and aflatoxin content where Aspergillus species were present in over 80% of the samples and aflatoxin B1 has been identified in almost 30% of the samples, mainly corn with the low concentrations <10 µg/kg. Braicu et al. (2008) investigated forty-three samples of different cereals (wheat, maize, rye and Triticale). It was found that 58.1% of samples were contaminated with different mycotoxins in various concentrations: aflatoxin B1 (1.6–5.7 µg/kg), aflatoxin B2 (0.89–4 µg/kg), aflatoxin G1 (1.2–5.76 µg/kg), aflatoxin G2 (0.96–3.4 µg/kg) and/or 4.3–30 µg/kg ochratoxin A. The concentration of total aflatoxin contamination ranged from 11.2 to 10.8 µg/kg. Among the different cereals, the highest number of contaminated samples was found to be in the wheat samples (62.5%). Šegvić Klarić et al. (2009) analyzed 37 samples of cereals and feed randomly collected in 2007 from households of an endemic nephropathy area in Croatia. The incidence of aflatoxins was 24.3 % with the average value of 4.6 µg/kg.

Conclusion

Based on everything stated above, it can be concluded that the presence of aflatoxin was highest in complete feeding mixtures for fattening pigs (43%), while in complete feeding mixtures for pregnant sows, aflatoxin was detected in only one sample with concentration of 14 μg/kg. This may be explained by smaller amount of maize used for preparation of complete feeding mixtures for pregnant sows. However, there was no information about their actual composition, so this is just a theoretical assumption. Contamination levels were above the maximum limit established by the European Union and Serbian regulations in 14 (13%) samples which can be characterized as quite high contamination. Since Serbian regulations for control of mycotoxins in feed was harmonized with EU regulations in 2014 it can be assumed that in the future there will be more frequent controls of aflatoxins and more data about its occurrence in Serbia.

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References

1. Bernardo F 2004. Micotoxicoses: Gestão de Risco. Prova de agregação. Faculdade de Medicina Veterinária, Lisboa, 172 pp.
2. Bertuzzi T, Pietri A, Barbieri G and Piva G 2003. Aflatoxin residues in milk of sows fed a naturally contaminated diet. Italian Journal of Animal Science 2, 234-236.
3. Braicu C, Puia C, Bodoki E and Socaciu C 2008. Screening and quantification of aflatoxins and ochratoxin A in different cereals cultivated in Romania using thin-layer chromatography-densitometry. Journal of Food Quality 31, 108–120.
4. Commission Directive 100/2003 of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Official Journal of the European Union, L 285, 33-37.
5. Commission Regulation 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, L 70, 12-34.
6. Crenshaw M 2008. Mycotoxin in Swine Diets. Retrieved December 17, 2013, from: http://en.engormix.com/MA-pig-industry/health/forums/article-mycotoxin-swine-diets-t4093/165-p0.htm.
7. Jajić I, Krstović S, Perišić B and Glamočić D 2013a. Presence of aflatoxin in complete feeding mixtures for milking cows in Serbia. 23rd International Symposium „New technologies in contemporary animal production", Novi Sad, Serbia, 28 pp.
8. Jajić I, Perišić B, Krstović S and Kos J 2013b. Aflatoxins – maize safety economy challenge in 2012. Savremena poljoprivreda 62 (3-4), 133-138.
9. Kos J, Janić Hajnal E, Mastilović J, Milovanović I and Kokić B 2013a. The influence of drought on the occurrence of aflatoxins in maize. Proceedings for Natural Sciences Matica Srpska 124, 59-65.

10. Kos J, Mastilović J, Janić Hajnal E and Šarić B 2013b. Natural occurrence of aflatoxins in maize harvested in Serbia during 2009-2012. Food Control 34, 31-34.
11. Kurtzman CB, Horn BWE and Hesseltine CW. 1987. Aspergillus nomius, a new aflatoxin-producing species related to Aspergillus flavus and Aspergillus parasiticus. Antonie van Leeuwenhoek 53, 147-158.
12. Lanyasunya T, Wamae LW, Musa HH, Olowofeso O and Lokwaleput IK 2005. The Risk of Mycotoxins Contamination of Dairy Feed and Milk on Smallholder Dairy Farms in Kenya. Pakistan Journal of Nutrition 4, 162-169.
13. Liu CL, Wang P, Yang YH and Wu WG 2009. Effects of Aflatoxins on Reproduction in Sows. Journal of Hebei North University (Natural Science Edition) 3, 19.
14. Magnusson B, Näykki T, Hovind H and Krysell M 2012. Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories. Nordic Innovation, Oslo, Norway.
15. Martins LM 1989. Capacidade de produção de aflatoxinas por Aspergillus flavus em substratos naturais. Repositório de trabalhos do LNIV 21, 123-132.
16. Pravilnik o kvalitetu hrane za životinje. Službeni glasnik Republike Srbije, 27/2014. Retrieved May 5, 2014, from: http://www.e-glasnik.rs/SlGlasnikPortal/pages/home.xhtml.
17. Reason AJ 2003. Validation of Amino Acid Analysis Methods. In Methods in Molecular Biology: Protein Sequencing Protocols, (eds BJ Smith), pp. 181-194. Humana Press Inc., Totowa, NJ, USA.
18. Republic Hydrometeorological Service of Serbia 2012. Agrometeorološki uslovi u proizvodnoj 2011/2012 godini na teritoriji Republike Srbije.
19. Šegvić Klarić M, Cvetnić Z, Pepeljnjak S and Kosalec I 2009. Co-occurrence of aflatoxins, ochratoxin A, fumonisins, and zearalenone in cereals and feed, determined by competitive direct enzyme-linked immunosorbent assay and thin-layer chromatography. Arhiv za higijenu rada i toksikologiju 60(4), 427-433.
20. Shi YH, Fang LY, Sun Y, Xu ZR and Wang CZ 2007. Effects of aflatoxin on growth performance and liver function of pigs. Journal of Northwest A & F University (Natural Science Edition) 6, 11.
21. Stoloff L 1977. Aflatoxins – an overview. In Mycotoxins in Human and Animal Health (eds JV Rodncks, CW Hesseltine and MA Mehlman), pp. 7-28. Pathotox, Park Forest South, Illinois, USA.
22. Tabuc C, Stroia C and Neacşu A 2010. Incidence of Aspergillus strains and of aflatoxin B1 in cereals in south-western Romania. Agricultural Science Research Journal 42(2), 322-327.
23. United States Food and Drug Administration (FDA). 2000. Retrieved March 12, 2014, from: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm.
24. Van Egmond HP and Jonker MA 2004. Worldwide regulations for mycotoxins in food and feed in 2003. The Food and Agriculture Organization of the United Nations (FAO). Food and nutrition paper no. 81.
25. Veldman A, Meijs JAC, Borggreve GJ and Heeres-van der Tol JJ 1992. Carry-over of aflatoxin from cows' food to milk. Journal of Animal Production 55, 163-168.
26. Wood GE 1991. Aflatoxin M1. In Mycotoxins and Phytoalexins (eds RP Sharma and DK Salunkhe), pp. 145-164. CRC Press, Inc., Boca Raton, Florida, USA.
27. World Health Organization 1999. Basic Food Safety for Health Workers. 17-28.
28. Zain ME 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15, 129–144.