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CONTAMINATION OF CEREALS WITH AFLATOXINS, METABOLITES OF FUNGI *Aspergillus flavus*

D. Jakić-Dimić[1], K. Nešić[1], M. Petrović[2]

[1]Institute of Veterinary Medicine of Serbia, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: nivs@net.yu
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Abstract: In stored cereals besides other microorganisms, *Aspergillus flavus* fungi, well known aflatoxin producers, can also be found. When these feedingstuffs, as main material for feed production, are very contaminated with fungi and mycotoxins, they induce different health disturbances both in animals and humans. However, the presence of fungi is not a proof of mycotoxins contamination because thay are produced in specific conditions. By regulating this environmental situation, it is possible to prevent contamination with mycotoxins to a certain extant, as well as their expansion and their threat to health. In this paper results of microbiological and mycotoxicological examinations of 968 cereal samples (corn, wheat, bran, silage, barley, soybean, sorghum) are presented. Samples were sent from different localities in Serbia during 5 years period. At the microbiological examination of cereals it was noticed that 675 samples did not fulfilled legislative requirements regarding fungi content and the biggest difference was present in naturally dried corn. Usually were found *Aspergillus* spp., *Penicillium* spp., *Fusarim* spp., *Mucor* spp., while *A. flavus* was present in 154 samples. It was also noticed that aflatoxin was present in 149 samples up to 0,05 mg/kg, what was still in accordance with the actual regulations.

Key words: aflatoxins, cereals, fungi

Introduction

Although detrimental effects of mouldy food and feed have been well known since long time ago, fungi *Aspergillus flavus* and several unknown substances named aflatoxins were not determined until 60-ies of the last century. Few hundreds of micotoxins have been found so far, but only several are considered to be harmful *(Riley, 1998)* and 20-30 among them, according to the frequency of apperience and their adversity, have medical, nutritive, ecological and economic significance.

There is few categories of mycotoxins regarding their chemical structure, sensitivity of certain organs and origin of fungi that produce them. According to the effects on different organs they can be hepatotoxic, nephrotoxic, neurotoxic and cytotoxic *(Sinovec et al., 2000)*.

Aflatoxins have hepatotoxic activity and they represent a mixture of several chemical substances. They are heterocyclic secundar metabolites of *Aspergillus* fungi. Their synthesis depends on environmental conditions in which fungi grow, but its production is also dependant on the fungus strain *(Pasteiner, 1998)*. Although it is well known that aflatoxins are metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* only *(Diener and Daviš, 1966)*, there are certain strains of both fungi which are not toxin producers at all. It is noted that *A. parasiticus* often contaminates oilseeds and produces B_1, B_2, G_1 and G_2 aflatoxins, while *A. flavus* can usually be found at cereals and generates only B_1 and B_2 aflatoxins *(Mixon, 1977; Zuber, 1977)*. Strains of both *A. flavus* and *A. parasiticus* can make aflatoxin M_1, but there are also strains which do not produce any toxins.

Aspergillus flavus is fungus widely spread in nature which can be isolated from kernels of corn, wheat, barley, oats, rice, cotton seed, coffee, fish meal and many other feed stuffs. It is typical for peanut whish is infested underground, especially in tropical and subtropical regions.

Fungi produce aflatoxins in the presence of higher moisture, temperature and adequate substratum. Synthesis is highest when humidity is above 13% and temperature is between 24º and 37ºC. That is why warm and wet geographic regions are the most favorable environments for aflatoxins and usually are affected.

According to its frequency of occurrence and its toxicity aflatoxin B_1 is the most important among all in the group. Aflatoxicosis is manifested with different pathomorphological changes, especially in the liver, but also in kidneys, cardiovascular and nervous system. Moreover, it exerts detrimental effects on performances, primarily in broiler and layer production, but also in swine breeding and all other modes of animal husbandry. Residues can be found in the organs of animals which ingested contaminated feed in the liver, muscles, gaster, kidneys and fatty tissue, as well as in meat, milk and eggs *(Resanovic and Sinovec, 2005)*.

This paper presents results of examination of different cereals samples: corn, naturally dried and artificially dried, wheat, barley, soy beans, sorghum, silage and wheat bran which had been sent to laboratories of Institute of Veterinary Medicine of Serbia in Belgrade. The aim of this investigation was to show frequency of appearance of *Aspergillus flavus* at cereals and to identify level of contamination of cereals and some of their products with this fungus metabolites-aflatoxins.

Material and Methods

Microbiology analyses of samples originated from silo, animal farms and fish ponds were done with the aim to identify level of contamination of cereals, and some of their products, with fungi and their metabolites aflatoxins. All the data were collected during five years period. In total it was inspected 968 samples of cereals:

- corn - 443 samples
- wheat - 304 samples
- intermediate products of wheat grinding (bran) - 63 samples
- corn silage - 58 samples
- barley - 27 samples
- soya - 63 samples
- sorghum - 10 samples

Samples of corn, wheat and soya were taken from the silo cells, by droping grains from the bottom into polyvinil bags which were sent to laboratory as part of regular control in feed mills. Samples of cereals which came from farms were brought by Institute clinitians. Wheat bran samples were taken from the mills directly during techology of processing wheat. Sorghum for these analyses was received in original packs as bird feed.

All samples were prepared for micology tests in accordance to procedure of accredited method in the Institute's laboratory for feed microbiology. Defined amounts of inoculum were transfered into Petry dishes applying pour-plate technique for adding Saburo agar. After 1-14 days of incubation at 28°C microscopic examination of grown fungi colonies was done and afterwards their isolation and determination.

Aflatoxins presence, produced by isolated strains of *A. flavus*, was detected by ELISA method which was based on antigen-antybody reaction. Into the wells of microtitar plate, according to directions of commercial kit (R-Biopharm: Aflatoxin total) standards and samples were introduced. To make reaction visible chromogen had to be supplemented, which in contact with enzymes became bly, but after addition of stop reagent it became yellow. At the end adsorbance was measured fotometricly at the ELISA reader and, concidering the standard curve, calculation of mycotoxins content in the sample was done precisely. All results were compared and interpreted regarding national Directive on maximal amounts of harmful materials in feed *(Službeni list SFRJ, 1990)*.

Results and Discussion

At the surface of stored grains certain amount of different microorganisms is always present. Microflora of wheat, corn, barley seeds mostly consists of bacteria, primarly genera: *Pseudomonas, Bacillus, Proteus.* But also fungi genera: *Mucor, Rhizopus, Aspergillus, Penicillium, Alternaria, Fusarium*, and very few actinomycetes, could be found.

At the inspection of received samples different species of bacteria and fungi vere isolated. But, the main concern was mycology examination which showed that 675 samples did not fulfilled legislative requirements. Determination of *A. flavus,* as a dominant producer of aflatoxins in cereals, was the aim of analysis. Results are showen in Table 1.

Table1. Results of mycology and mycotoxicology examination of cereals

Sort of samples	Number of samples	Samples with *A. flavus* infestation	Samples contaminated with aflatoxins 0-0.05 mg/kg (ppm)
1. corn	443	83	81
2. wheat	304	61	58
3. bran	63	6	6
4. silage	58	0	0
5. barley	27	0	0
6. soya	63	0	0
7. sorghum	10	4	4
TOTAL:	968	154	149

Amongst 968 samples which were analysed in total, there were 443 corn samples. From 83 of them was isolated *A. flavus*, while presence of *A. parasiticus* was not noted, as well as in all other sorts of cereals. This showes that corn seeds, especially damaged, are suitable medium for fungi growth. Moulds rise at the corn both at the field and during storage. In storage conditions when moisture do not exceed 13% their growth is in stagnation. But, when humidity and temperature increase and mechanical damages of seeds are already present, fungi can rapidly grow and multiplicate. Some of these fungi then can also produce mycotoxins, which can be transfered with cereals, as main feedingstuffs, into the animal organisms and can cause different diseases and health disturbances.

Also 304 samples of wheat were analysed and *A. flavus* was isolated from 61 samples what represents 20%. Isolated strains of this fungus were identified as toxin producers because in almost identical percentage samples were recognized as aflatoxin positive

Wheat is plant which can be processed in different eatable products, but mycotoxins might also become part of them. Among all 63 analysed samples of

intermediate products of grinding *A. flavus* was determined in only 6 samples of bran which also showed aflatoxin presence, so it was concluded that all isolated strains were toxin producers.

Higher percentage was obtained for 10 sorghum examinations which were in 4 samples contaminated with *A. flavus* and aflatoxins at the same time.

But the presence of fungi itself does not necessairly confirm mycotoxins contamination. Certain fungi which are toxin producers can be found in material, while mycotoxins are absent because there is an evidence that the mycotoxins production only happens in specific environmental requirements. Our results also confirm this theory because not in all samples contaminated with *A. flavus* aflatoxins were found simultaneously. Aflatoxin in them was detected in amount up to 0.05 mg/kg (ppm), which is is the highest permited amount for feedingstuffs according to national Directive on maximal amounts of harmful materials in feed *(Službeni list SFRJ, 1990)*.

Improvement of storage conditions: increasing moisture and better ventilation, can reduce risk of mycotoxins syntesis in cereals representing one of the most important steps in prevention of this problem, besides other fisical, chemical and nutritive treatments for crops at the field to complete feed production.

Conclusion

According to obtained results of examination of different cereals and some of their products, collected during five years period from various localities in Serba, stored in silos, at the farms and fish ponds, it can be concluded:

- Stored corn was mostly infested with *A. flavus*
- At the stored wheat grains, besides the other microflora, certain amount of *A. flavus* was also identified with the ability to produce mycotoxins
- Contamination of wheat grains during processing can be transfered to its products
- Isolated strains of *A. flavus* showed the ability to produce aflatoxins in adequate conditions
- In 97% of samples with *A. flavus* infestation aflatoxin was also detected, but in the amount below maximal limit prescribed in national Directive *(Službeni list SFRJ, 1990)*.

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Kontaminacija žita aflatoksinima, metabolitima plesni *Aspergillus flavus*

D. Jakić-Dimić, K. Nešić, M. Petrović

Rezime

Na osnovu rezultata mikrobiološke i mikotoksikološke analize 968 uzoraka žita (kukuruz, pšenica, mekinje, silaža, ječam, soja, sirak) koji su pristizali u ovlašćenu laboratoriju na kontrolu sa teritorije Republike Srbije u toku petogodišnjeg perioda utvrđeno je da sadržaj gljiva (plesni) kod 675 uzoraka žita ne odgovara uslovima Pravilnika o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani *(Službeni list SFRJ, 1990)*, a najveći stepen odstupanja utvrđen je u prirodno sušenom kukuruzu. Determinacijom gljiva najčešće izolovane vrste su: *Aspergillus* spp., *Penicillium* spp., *Fusarim* spp., *Mucor* spp., a u 154 uzoraka identifikovano je prisustvo vrste *Aspergillus flavus.* Ispitivanjem sadržaja aflatoksina, u 149 uzoraka žita, ustanovljen je aflatoksin do koncentracije 0,05 mg/kg (ppm), što je u granicama dozvoljenim važećom regulativom.

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