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DEGREE OF MYCOTOXICOLOGICAL CONTAMINATION OF FEED AND COMPLETE FEED MIXTURES FOR PIGS AND POULTRY DURING THE PERIOD 2007–2012. ON THE TERRITORY OF THE REPUBLIC OF SERBIA

ABSTRACT: The most common producers of mycotoxins are fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium*. Toxins are of extreme importance because it can be transmitted from animals to humans through milk and animal products, some of which are carcinogenic and teratogenic. Mycotoxins cause a health disturbance of all animals, but the effects are more noticeable in highly productive animals in the farm way of keeping considering the much greater consumption of concentrate feeds, although forages also can be contaminated with mycotoxins in a significant manner. Mycotoxicoses are the most common seasonal illnesses, and are an important diagnostic problem in veterinary practice, because its characteristics often resemble diseases caused by pathogens or nutritional deficiency or imbalance. The degree of health disturbances depends on the amount of toxins in feed and the length of intaking as on types and categories of animals.

The presence of mycotoxins in animal feed is inevitable and therefore testing of raw materials and products is necessary so that feed for humans and animals can be safe for use. Damages arising as consequences of mycotoxicosis in poultry and swine production, due to the direct loss because of animals lossor, more commonly, indirectly due to the fall productive and reproductive performances of animals, imposed the need for continuous monitoring of the hygienic quality of feed mixtures for feeding these animals.

During a five year period (2007-2012) were analyzed a total of 104 samples from the territory of Republic of Serbia intended for nutrition of all categories of poultry and mixtures for the initial and final fattening broilers (50 samples) and laying hens (54). The analysis included 57 samples of feed mixtures intended for all categories of swine – feed mixture for young (20 samples) and the old categories (37 samples) and 196 of the samples, which are commonly used in formulating rations for listed species (maize, soybean and sunflower meal). For the analysis of the samples was used thin layer chromatography and Elisa test. The results were compared with current Regulations on the quality of the feed

(Official Gazette of RS 41/09) in force since 1.05.2010. in the part where the maximum allowable quantities of hazardous substances (Article 99) gives the value of the maximum permitted levels of mycotoxins in animal feed. The number and types of mycotoxins vary depending on the feed, as well as on the year which can be directly related to climatic factors, and the average annual humidity. The fact that in the samples was not detected the presence of aflatoxin indicate that in our conditions toxin producing fungi do not find fertile ground for the production of toxins, as well as the absence of certain nutrients in the production of complete feed mixtures for pigs and poultry, which are the traditional sources of aflatoxin (peanut oil meals). The results are encouraging given the fact a relatively small number of defective mixtures and nutrients. However, the fact that only a limited number of feed samples we received for the analysis suggests further caution and constant monitoring of the presence of mycotoxins in animal feed.

KEY WORDS: Mycotoxins, pigs, poultry, feed

INTRODUCTION

Mycotoxins are secondary toxic metabolites of a number of saprophytic molds that enters the body of animals and humans mostly through contaminated feed infested with spores, conidia and / or mycelium fragments. When toxins get into the body of animals and humans they cause intoxication, so called mycotoxicoses, given that they are related to feed, can take on a wide scale (U r a g u c h i and Y a m a z a k i, 1978).

It is assumed that the mycotoxins were present in food for animals and people from the beginning of life on Earth, and the first data on the harmful effects of the consumption of mycotoxins in China date back to 5000 years ago. Although the harmful effects of animals and humans nutrition with moldy food were well known since ancient times, a specific agent was not known for a long time. The emergence of unknown diseases (“Turkey X disease”), which is in England 1960th led to the deaths of more than 100,000 turkeys and about 20,000 other types of birds with signs of acute liver necrosis (L a n c a s t e r et al., 1961, B u t l e r, 1974), directed the research on determining the causal factors. A year later, from the imported peanut meal, raw material used for poultry feeding, was isolated culture of fungi *Aspergillus flavus*, as well as a few previously unknown compounds, which fluoresced very intense under UV light.

The discovery of these compounds, called aflatoxins, represents a milestone in the history of mycotoxins. Researchs over the past 40-odd years accumulated many data so that until now have been revealed several hundred mycotoxins (S m i t h and M o s s, 1985), of which only smaller number has been considered harmful (R i l e y, 1998), and only 20-30, by the incidence and adverse effects has medicinal, nutritional, environmental and economic importance. It is known that over 220 species produces mycotoxins and most of the toxin-producing species belongs to the genera of *Fusarium*, *Penicillium* and *Aspergillus*. It is known that mycotoxicoses are cause-effect related to the occurrence of a few very large-scale poisoning and death of hundreds of thousands of animals and people in Europe and other continents in the previous millennium (U e n o, 1983).

Contamination with molds and mycotoxins is a current global problem, according to FAO data today is about 25% of cereal production globally con-

taminated (Devegowda et al., 1998). It is evident that molds and mycotoxins are a serious problem (SCOPP, 1996), not only in terms of the success of the harvest and grain quality, but also in relation to animal health and productivity (Mašić et al., 2002b), and safety of feedstuffs of animal origin, aspects of health (Miller and Trenholm, 1994). It is believed that the economic losses caused by mycotoxins are almost immeasurable (Devegowda and Aravind, 2002).

Damage to livestock caused by mycotoxicosis manifest themselves in the form of direct losses due to loss of animals or, more commonly, indirectly due to falling productive and reproductive performances of animals. Mycotoxins cause a health disturbance of all animals, but the effects are more noticeable in highly productive animals in the farm way of keeping in view of the much greater consumption of concentrated feed even though, forages may be contaminated with mycotoxins in a significant manner (Pasterner, 1998, Fink-Gremmels, 2005). Diseases caused by mycotoxins are not contagious, they are related to feed and / or specific nutrients, similar to vitamin deficiencies are not treated with antibiotics or other drugs, in the body do not cause an immune response, because they are low molecular weight, and the animals are permanently unprotected from their effects (Wyllie and Morehouse, 1977). Mycotoxicoses are the most common seasonal illnesses, and are an important diagnostic problem in veterinary practice, because by its characteristics often resemble the diseases caused by pathogens or nutritional deficiency or imbalance. Poisoning are manifested in the form of primary acute or chronic toxicosis, as well as in the form of secondary toxicosis (Richard and Hurston, 1986; Nured and Riley, 2001).

Changes caused by mycotoxins depend on the type and quantity of mycotoxins in feed, the length of ingestion, as well on genetic (species, breed, animal strain), physiological (gender, category, age, diet) and environmental (climatic conditions, keeping animals) factors, and the presence of disease infectious and / or noninfectious nature (Smith and Moss, 1985).

A particular issue is possibility that in the body of animals that consumed feed contaminated with mycotoxins can be found a mycotoxins in various amounts, so there may be a manifestation of adverse effects in humans. (Ožegović and Hlubna, 1981, Ožegović, 1983). There must not be lost from the sight that main goal of feed production is to ensure the health of people who consume feeds of animal origin, and only then to meet the nutritional requirements of animals and the preservation of their health. Damages in the poultry and swine production caused by mycotoxicosis, direct losses due to loss of animals or, more commonly, indirectly due to falling productive and reproductive performance of animals, imposed the need for continuous monitoring of the hygienic quality of feed mixtures and different feed used in formulating rations for feeding these animals.

Considering the frequency of occurrence in the feed, in conjunction with conditions on our geo-climatic region, in the most important fungi can be classified *Aspergillus* and *Fusarium* species that contaminate feed in the fields and warehouses.

Aspergillus mycotoxins

This group contains a number of mycotoxins (sterigmatocystin, citrulline, patulin), but aflatoxin B1 (AFB1) and ochratoxin A (OTA) are certainly the most important representatives of this group.

Aflatoxins

Aflatoxins, according to the structure of molecules, belong to a group of heterocyclic derivatives of bisfuranokumarin type, and aflatoxin B1 (AFB1) is the most toxic mycotoxin for both human and animal. In terms of aflatoxin toxicity with implications for human health certainly the most important metabolite is aflatoxin M1, while aflatoxin M2 and M4 are of minor importance (Gorelik, 1990).

The most common way of the aflatoxin absorption is through gastrointestinal tract, lungs and skin. Transport of chemicals through the cell membrane is in direct correlation with their liposolubility (Klassen and Rossman, 1991).

Aflatoxin tends to be deposited in all soft tissues and fat depots of animals. The highest level of aflatoxin accumulation occurs in tissues which serve its biotransformation, such as the liver and kidneys (Lesson and Summers, 1995). Aflatoxins ingested by feed pass the gastrointestinal tract and reach the bloodstream within 30 minutes, and in the liver for 1 hour. Biodegradation of aflatoxin molecules in hepatocytes occurs in at least six ways (Ueno, 1983), and is considered among the metabolites of aflatoxin B1, aflatoxins to be the most mutagen agent that causes cancer changes in the liver.

Aflatoxin residues can be found in the tissues and organs, as well as eggs from laying hens and milk from animals fed with aflatoxin contaminated feed (Lesson and Summers, 1995). Aflatoxins are detectable in all parts of the egg not before 10 hours after ovulation. The amount of aflatoxin decreases in egg white after 48 hours, while the content in the egg yolk and shell increases (Jacobson and Wisman, 1974). Aflatoxins, particularly AFB1, are highly toxic compounds (LD50 1-50 mg/kg) and in addition to acute toxicity exerts a very strong carcinogenic effect (Eaton and Groopman, 1994). The International Agency for Research on Cancer (IARC) has classified AFB1 in group 1 of carcinogens, because the risk of possibility of primary human liver cancer is very high (Henry et al., 2001, 1999).

Ochratoxins

Ochratoxins, of which the most important type A (OTA), are by the chemical structure isocoumarin derivatives (Bertina, 1984) and are absorbed relatively slowly from the digestive tract (Uraguchi and Yamazaki, 1978). High affinity of OTA to plasma proteins is an important factor that facilitates passive absorption of the non-ionized form of toxins from the digestive

tract, but also makes difficult its elimination from the organism by limiting glomerular filtration and renal excretion.

In the liver OTA hidrolisis forming the less toxic metabolites. Excreted via the bile into the intestine, and are subject to reabsorption (S i n o v e c et al., 1998). Feces excretion is 11% of unchanged OA, and about 23-33% metabolised mycotoxins in the form O α .

Excretion is done efficiently and throughout urine (11% unchanged OTA), but OTA is subject to reabsorption in the renal tubules, which is the basis of residual effect of mycotoxins in the kidneys, and probably in the whole body (F u c h s, 1988). It is characteristic that mercury has the ability of efficiently and faster excretion of mycotoxins so that over a 90% of ingested OTA is excreted during 48-h (G a l t i e r et al., 1981).

There is a significant possibility of depositing OTA and its metabolites in the edible parts of pigs and poultry (M a s i c, 1986; Z u r o v a c - K u z m a n, 2001) especially in the kidneys and liver (F u c h s, 1988), and considerably lesser in muscle and adipose tissue (J o n k e r et al., 1999). Scandinavian countries introduced mandatory inspection of meat and kidney of pigs slaughtered in abattoirs, and WHO has prescribed the maximum permissible concentration of OTA in meat of slaughtered animals. Analyses of meat can show the presence of OTA residues in a large number of apparently healthy animals (up to 25%), and similar results were obtained in previous studies (M i l i c e v i c, 2004).

Fusarium mycotoxins

Fusarium mycotoxins are commonly identified group of mycotoxins in feed. Some strains of Fusarium fungi can produce up to 17 mycotoxins at a time, and in this group, beside zearalenone (ZON) and T-2 toxin are classified other trichothecenes fall, fumonisins, moniliformin and fuzaric acid.

Zearalenone

Zearalenone (F-2) belongs to a group of phytoestrogens and untill now has been identified 15 different products that have different biological activity (B e t i n a, 1984). Basically, they have a similar configuration (phenolic core) to estrogenic substances (estradiol, estriol and stilbestrol).

Zearalenone after the oral ingestion very well and quickly absorbs. It can be found in the plasma after 30 minutes from the moment of feed intake in the organism of pigs (O l s e n et al., 1981). It is believed that the F-2 is from less toxic than other metabolites of Fusarium fungi, and the relative toxicity (LD50) ranges from 1-10 mg/kg (T e r a o and O h t s u b o, 1991).

Most of the absorbed zearalenone is transported by the portal blood stream to the liver (U r a g u c h i and Y a m a z a k i, 1978), where is accumulated and metabolized by enzymes (reductase and esterase) creating more (up to 4 times) or less active metabolites than its predecessor. F-2 and its metabo-

lites are distributed primarily by target tissues as the uterus, intestine, testes, ovaries, and adipose tissue (Riley, 1998).

F-2 residues and the resulting products can be determined in the edible parts of the animals feeding with contaminated feed, mostly in the liver and muscles (and Ciegler Vesonder, 1983), but also in milk and eggs. Even in meat of clinically healthy animals may be determined the quantity of residues up to 10 mg/kg. The chickens meat is deposited with large amounts of metabolites (59-1200 mg/kg) more than in the meat of pigs with lower feed contamination (78-310 mg/kg). Residues are carcinogenic, and their biological effects are compared with the effects of dietilstilbestrola or estradiol.

MATERIALS AND METHODS

During a five year period (2007-2012) was analyzed a total of 104 samples from the Republic of Serbia intended for nutrition of all categories of poultry – mixtures for the initial and final fattening broilers (50 samples) and mixtures for laying hens (54).). For the samples analysis were used thin layer chromatography and ELISA tests. The study included 57 samples of feed mixtures intended for nutrition of all categories of pigs – feed mixtures for young (20 samples) and the old categories (37 samples) and 196 of the samples, which are commonly used in formulating rations for listed species (corn, soybean and sunflower meal). The results were compared with current Regulations on the quality of the feed (Official Gazette of RS 41/09) in force since 1.05.2010. and in the part where the maximum allowable quantities of hazardous substances (Article 99) gives the value of the maximum permitted levels of mycotoxins in animal feed (table 1).

RESULTS OBTAINED

From the total number of analyzed samples of feed mixtures intended for feeding all categories of pigs (57) there were 20 samples of feeding mixtures for young and 37 for elderly categories. The content of aflatoxin in feed mixtures for young varied in the range from 0.001 to 0.0092 mg/kg and a similar trend was noted for the adult category with a range of 0.001 to 0.018 mg/kg. Comparing the results with the actual national maximum allowable values for aflatoxin conclusion is that none of the samples were found above the permissible value.

The recorded values of zearalenone in feed mixtures for feeding young pigs varied in the range of 0.006 to 2.786 mg/kg. Of the total number of feed mixtures samples for feeding young (20) in the two samples the presence of zearalenone was above permitted level in the quantity of 2.786 and 0.819 mg/kg, which in the aggregate make up 10 percent of the total samples analyzed for this group of pigs and 3.51 % based on the total number of feeding mixtures samples intended for pigs (chart 1).

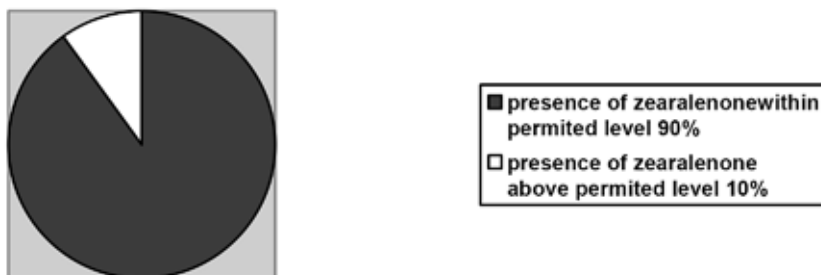


Chart 1 –
Samples of feeding mixtures intended for pigs nutrition with the presence of zearalenone above and within permitted level

Analysis of feeding mixtures for pigs adult categories established values for zearalenone ranged from 0.01 to 0.35 mg/kg, which corresponds to the allowed values.

In the analysis of feeding mixtures for feeding young pigs established values for ochratoxin ranged from 0.001 to 0.10 mg/kg, which is consistent with the allowable values prescribed by regulation. The determined values for ochratoxin in feed mixtures for adult pigs categories were in the range from 0.002 to 0.2 mg/kg, also in accordance with the allowable values prescribed by regulation.

Total mycotoxicological analysis included 104 samples of feed mixtures intended for all categories of poultry : 50 samples of feed mixtures for the initial and final fattening of broilers and 54 samples of laying hens. The content of aflatoxin in feed mixtures for broilers varied in the range of 0.001 to 0.03 mg/kg and for adult categories ranging from 0.001 to 0.009 mg/kg. Comparing the results of the samples of feed mixtures for laying hens with applicable regulations of maximum allowable values for aflatoxin was found that none of the samples had detected values above the allowable. However, the determined value for aflatoxin in samples of feed mixtures for the initial and final fattening of broilers at five samples exceeded the allowed values which in the aggregate makes 10 percent of the total samples analyzed for this category of poultry or 4.81% based on the total number of samples of feed mixtures intended for poultry nutrition (chart 2).

In the analysis of feed mixtures for broilers detected values for zearalenone ranged from 0.013 to 1.257 mg/kg and for laying hens they were 0.013 to 0.54 mg/kg. The current regulations do not regulate the maximum permissible values of these mycotoxins in complete and supplementary feed mixtures for livestock.

The determined values for ochratoxin in feed mixtures for broilers ranged from 0.002 to 0.65 mg/kg, which is within the prescribed values same as the values determined in feed mixtures for laying hens from 0.004 to 0.10 mg/kg.

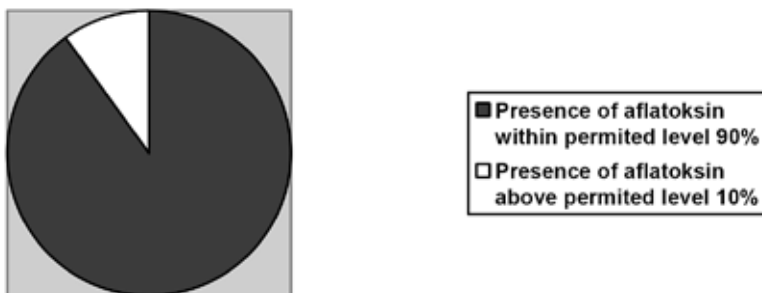


Chart 2 –
Samples of feeding mixtures intended for poultry nutrition with the presence of aflatoxin above and within permitted level

During the mucotoxicological analysis of feeds we chose those materials that are widely used in pig and poultry diets and commonly used in formulating their meals: corn, soybean and sunflower meal. From a total of 196 samples of feeds not in one examined sample has been detected value of aflatoxin in excess of allowable values prescribed in the regulation except that the two nutrients had the maximum allowed value of 0.05 mg/kg. Presence of zearalenone was detected in the range of 0.001 to 0.62 mg/kg and ochratoxin 0.001 to 0.30 mg/kg with notice that currently applicable regulations do not regulate the maximum permissible value of these mycotoxins in feed, but only in complete and supplementary feed mixtures.

Tab. 1 – Maximally permissible levels of harmful substances (Official Gazette of RS 41/09 Article 99)

Type	Feed and feeding mixtures	mg/kg (ppm)
Aflatoxin	Feeds	0,05
	Complete and supplemental mixtures for cattle, sheep and goats, with the exception of dairy cows, calves, lambs and kids	0,05
	Complete and supplemental mixtures for dairy cows	0,01
	Supplemental mixtures for pigs and poultry except offspring	0,03
	Complete and supplemental mixtures for pigs and poultry except offspring	0,02
	Complete and supplemental mixtures for calves, lambs, goats, pigs, chickens, little girl, ducklings	0,01
Zearalenone and its derivatives	Complete and supplemental mixtures for pigs, gilts to 50kg body weight	0,50
	Complete and supplemental mixtures for other categories of pigs	1,0
	Complete and supplemental mixtures for cattle, sheep and goats	3,0
Ochratoxin A	Mixtures for pigs	0,1
	Mixtures for fattening pigs and breeding sows	0,2
	Mixtures for poultry	1,0
	Mixtures for layers	0,25

DISCUSSION

Aflatoxin is, in our country during the period of 1999-2000. year, determined in amount of 20.1-21.6% of analysed samples of animal feed with an average content of 0:05 to 00:04 mg/kg, and some samples contained even 0.10 mg/kg (Bočarov - Stančić et al., 2000a). Tryals performed in 2002nd (Mašić et al., 2003) have showed that out of 585 samples 10.43% was contaminated with AFB1.

By analyzing the results of mycotoxicological analyses of 78 samples for poultry in the period 1990-1994. year (Shaffer et al., 1994a) was obtained that 6.41% of the samples for broilers (5/78) contained aflatoxin in allowable values, while in the same period this toxin was not detected in samples of feed for layers (0/62). In the next period (1994-1996.), the situation was much better because none of the 16 samples of feed for broilers and 27 samples of hens did not contain aflatoxin above the allowable limit (Shaffer et al., 1997, 1998). However, in the period of 1997-2003 (Nedeljković - Trailović et al., 2004) even 24.44% of poultry feed samples contained AFB1 above the allowable limit.

Retrospective analysis of results of mycotoxicological tests performed on 74 feed mixture for chickens and 88 mixtures for laying hens in the ten-year period (1995-2004.) showed that 17.6 and 18.2% of the samples contained AFB1 in amounts of 0.05 ± 0.04 and 0.04 ± 0.03 mg/kg, respectively, and that of the contaminated samples, 76.92% and 93.75 contain tested toxins above the allowable limit (Sinovec, 2005).

By analyzing the results of mycotoxicological examination of 87 feed mixtures for pigs, 35 for fattening pigs and 36 for breeding pigs in the ten-year period (1995-2004) has been demonstrated that 26.4, 22.9 and 25.0% of the samples contained AFB1 in quantities of 0.05 ± 0.02 , 0.06 ± 0.04 and 0.06 ± 0.04 mg/kg respectively, and that 56.5, 100.0 and 22.2% of the contaminated samples contain tested toxins above the allowable limit (Marković et al., 2005). The number and types of mycotoxins vary depending on the feed, as well as on the year which can be directly related to climatic factors, and the average annual humidity. The fact that the results of the tested samples, presented in this paper, showed no significant presence of aflatoxin, suggests that the toxin producing fungi in our environment do not find fertile ground for the production of this toxins, as well as the absence of certain nutrients in the production of complete feed mixtures for poultry and pigs, which are the traditional sources of aflatoxin (peanut oil meal). However, detected values for aflatoxin in samples of feed mixtures for the initial and final fattening of broilers were at the five samples above the allowed values which in the aggregate make 10 percent of the total analyzed samples for this category of poultry or 4.81% based on the total number of samples of feed mixtures intended for poultry feeding, suggesting the need for permanent monitoring and caution in nutrition of given poultry categories.

Ochratoxin (OTA) has a special importance because of its ties with Balkan endemic nephropathy of people, which is a chronic disease with a fatal

ending (R a d o v a n o v i c, 1991). In some trials (R a d i c et al., 1986) it was found that 56.6% of the tested serum samples obtained from people of the West nephropathic area Posavina were positive for the presence of ochratoxin A. OTA is classified as potential carcinogenic for a population of people (group B), because, with a high content of OTA in feed (and C i e g l e r V e s o n d e r, 1983), was noticed high incidence of renal adenomas and carcinomas (R a d o v a n o v i c, 1991). The presence of OTA, as a natural contaminant, was first found in a sample of corn. OTA content in the feed and feedstuffs is usually lower than the 50 mg/kg, but in the incorrect storage may be identified significantly higher levels (J u r i c et al., 1999). In warmer regions, a significant number of samples usually contain very low amounts of OTA (<1 mg/kg), although and significantly higher levels can be detected (S p e i j e r s and E g m o n d, 1994).

OTA occurs naturally as a contaminant of various types of plant products such as cereals, flour, coffee, spices, pulses and dried fruit (S t u d e r - R o h r et al., 1995; K u i p e r - G o o d m a n and S c o t t, 1989). It was also found in wine, beer and fruit juices as a result of the use of contaminated raw materials for their production (J o r g e n s e n, 1998; K u i p e r - G o o d m a n, 1996).

In our country, during 1999-2000. year, ochratoxin was found in 41.2% of samples with an average content of 0.06 to 0.08 mg/kg, and some samples contained even 0.10 to 0.32 mg/kg (B o č a r o v - S t a n č i ć et al., 2000a). Tests performed 2002nd (M a š i ć et al., 2003) showed that out of 585 samples 15.56% was contaminated with OTA. In the period of 1990-1994. (S h a f f e r et al., 1994a) feed for broilers contained ochratoxin within acceptable limits, while 51.51% of feed samples for layers had OTA contamination above the allowable limit. In the next period (1994-1996.), the situation was much more favorable, because none of the 16 samples intended for broilers did not contain ochratoxin above allowable limits (S h a f f e r et al., 1997, 1998), while in the same period, feed for hens was defective in 37% (10/27). This period was characterized by the presence of ochratoxin in amounts up to 0.25 ppm in 49, or 63% of feed samples for broilers and laying hens, 0.25 to 0.50 ppm at 38, and 26% of cases and the 0.50 to 1.00 ppm in 13, or 11% of the feed samples for broilers and laying hens, respectively. On the other hand, in the period of 1997-2003. (N e d e l j k o v i c - T r a i l o v i ć et al., 2004) even 33.33% of poultry feed samples contained OTA above the allowable limit.

Retrospective analysis of results mycotoxicological examinations of 74 feed mixtures for chickens and 88 mixtures for laying hens in the ten-year period (1995-2004.) found that 94.6 and 92.0% of the samples contained OTA in amounts of 0.26 ± 0.15 and 0.23 ± 0.12 mg/kg or that 10.00 and 46.91% of the contaminated samples contained tested toxins above the allowable limit (S i n o v e c, 2005).

By analyzing the results of 87 mycotoxicological examinations of feeding mixtures intended for pigs, 35 samples for fattening pigs and 36 for breeding pigs in the ten-year period (1995-2004) have shown that all samples contain OTA in amounts of 0.27 ± 0.23 , 0.31 ± 0.14 and 0.27 ± 0.11 mg/kg and that 100.0, 82.9 and 19.4% of the contaminated samples contain tested toxins above the allowable limit (M a r k o v i c et al., 2005).

In the current conditions, the presence of OTA is the only relevant parameter for judging the contamination of feed mixtures for poultry nutrition, considering he is the most toxic of all mycotoxins in poultry (Leeson et al.) In the presented work there was not in any of the tested sample determined data for ochratoxin that exceeded the maximum allowable values required by the applicable regulations. However, the analyses of feedstuff intended for pigs and poultry found maximum values of 0.30 mg/kg but currently applicable regulations do not regulate the maximum permissible value of these mycotoxins in feed, but only in complete and supplemental mixtures. If the same criteria for nutrients as well as for complete and supplementary feed mixtures for pigs, breeding sows, fattening pigs and hens, could be applied then the number of samples that exceed the maximum permitted level would be much higher. It should be taken into account that the test feedstuff: corn, soybean and sunflower meals are also the most common raw material in formulating rations for this species and categories of animals and that must as well be considered in their use, especially with significant levels of detected mycotoxins.

Given the toxicity and frequency of occurrence in feed, zearalenone (ZON) is one of the most important mycotoxins of *Fusarium* fungus which are very widespread in nature and are very common in our geo-climatic region. Corn is the most commonly affected by contamination (Kuper-Godman et al., 1987), and hybrid variety with long vegetation and high humidity at the time of harvest are suitable for mold growth. The amount of zearalenone in contaminated corn is very different and usually ranges on average from 2 to 4 mg/kg, although it may be 12 mg/kg and higher.

The incidence and degree of contamination of grain with zearalenone vary depending on the type of seeds, climatic conditions and the method of storage (Pozzi et al., 1995). Corn, wheat and barley are the nutrients that are most contaminated, while other types of feed grains seem to be less contaminated with small amounts of zearalenone. Generally, the mean value of ZON detected in barley are relatively high, low in wheat, while in corn they vary.

In areas of our country most often are contaminated feedstuffs for pig, especially maize, which may contain an average of over 10 ppm ZON (Bočarov - Staňić et al., 1997a). The feed samples showed the presence of zearalenone in 70.7% of samples at amount of 0.2 to 20 mg/kg (Bočarov - Staňić et al., 2000b). During the 1999. and 2000. the presence of zearalenone was detected in 72.3 and 74.5% of samples with an average content of 0.66 or 2.39 mg/kg, but some of the samples contained toxin at 3.2, or 12.8 mg/kg (Bočarov - Staňić et al., 2000a). Trials performed 2002nd (Mašić et al., 2003) showed that out of 585 samples 15.04% were contaminated with ZON.

By analyzing the results of mycotoxicological examinations of 78 samples for poultry during the period 1990-1994. (Shaffer et al., 1994a) was obtained that 43.6% of the samples for broilers (34/78) and 51.6% samples for hens (32/62) contained ZON in allowed values. Also, in the next period (1994-1996.), none of the 43 poultry feed samples contained ZON in quantities above permissible (Shaffer et al., 1997, 1998), but the contamination in some samples was very high (0.72-10.70 ppm).

Retrospective analysis of results obtained from mycotoxicological examinations of 74 feed mixture for chickens and 88 mixtures for laying hens in the ten-year period (1995-2004..) showed that all samples contained ZON in amounts of 2.69 ± 5.14 and 5.29 ± 2.61 mg/kg, respectively within the permissible limits (Marković et al., 2005).

Testing the content of mycotoxins in feed for pigs (212 samples) in 2000-2001 years (Mašić et al., 2002a), it was found that the contamination of a mixture intended for young animals with unpermitted levels of mycotoxins (70.2%) was almost identical to the same for adult animals (69.1%). It is pointed out that the most common cause of feeding mixtures contaminations is related to the presence of mycotoxin zearalenone (an average of about 10.7 mg/kg of feed DM). The results indicate a significantly higher contamination of mixtures in comparison to previous studies carried out in the period 1988-1993. (Shaffer et al., 1994b) where was found to be 97.6% of samples of feeding mixtures for young contaminated with a zearalenone, or 81.0% of the samples for adult animals.

Retrospective analysis of results of mycotoxicological examinations 87 feeding mixtures for pigs, 35 for fattening pigs and 36 for breeding pigs during the ten-year period (1995–2004.) showed that all samples contained ZON in quantities of 5.06 ± 2.74 , 3.97 ± 2.33 and 5.25 ± 3.20 mg/kg and that 94.3, 85.7 and 94.4% of the contaminated samples contain tested toxins above the allowable limit (Marković et al., 2005).

In this work examination of feed mixtures for broilers established for zearalenone values ranged from 0.013 to 1.257 mg/kg and for the hens from 0.013 to 0.54 mg/kg. The current regulations do not regulate the maximum permissible value of these mycotoxins in complete and supplementary feed mixtures for poultry. Relatively high amount of zearalenone in the feed mixtures samples for poultry nutrition does not need much to concern in view of the higher resistance of poultry compared to other species, but suggests caution because it as an indicator of ongoing contamination of complete feeding mixtures by toxin producing fungi. On the other hand the presence of zearalenone in feed as well in a complete feed mixture for pigs is a permanent threat in view of their sensitivity to this mycotoxin and a wide spectrum of clinical manifestations that Zearalenone causes. Of the total number of feed mixtures samples for young (20), examined in this paper, for the two samples the presence of zearalenone was above permitted level and the quantity was 2.786 and 0.819 mg/kg, which in the aggregate make 10 percent of the total analyzed samples for this group of pigs or 3.51% based on the total number of feeding mixture samples intended for pig nutrition.

CONCLUSION

Generally speaking, the results are encouraging in view of the fact that relatively small number of faulty mixture was detected. However, the fact that only a limited number of feed samples we received for analysis and which

were already during the sensory examination suspicious for the presence of mycotoxins, as well as the history and clinical picture of animals fed with examined feed, suggest caution and mycotoxin presence. Early or timely determination of the presence of mycotoxins in feed and subsequent elimination from the use of contaminated feed and / or possible dilution or mixing with feed free of mycotoxins can mitigate the negative effects, but it requires a certain period of time for elimination of resorbed quantities of mycotoxins and adverse effects. Therefore, in the production conditions it must be practiced continuous and multistage monitoring of hygienic quality of feed in order to respond quickly and efficiently as, currently, the only successful way to prevent harmful effects of mycotoxins.

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СТЕПЕН МИКОТОКСИКОЛОШКЕ КОНТАМИНАЦИЈЕ ХРАНИВА И ПОТПУНИХ КРМНИХ СМЕША ЗА ИСХРАНУ СВИЊА И ЖИВИНЕ ТОКОМ ПЕРИОДА 2007–2012. ГОДИНЕ НА ТЕРИТОРИЈИ РЕПУБЛИКЕ СРБИЈЕ

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Резиме

Микотоксине најчешће производе гљивице из родова *Aspergillus*, *Fusarium* и *Penicillium*. Токсини су од изразите важности јер се могу пренети са животиња на људе путем млека и животињских производа, а неки од њих су канцерогени и тератогени. Микотоксини доводе до поремећаја здравственог стања свих животиња, али су ефекти уочљивији код високо производних животиња у фармском начину држања с обзиром на знатно већу конзумацију концентрованих хранива иако и кабаста хранива могу да буду контаминирана микотоксинима у значајнијем степеноу. Микотоксикозе су најчешће обољења сезонског карактера, а представљају значајан дијагностички проблем за ветеринарску праксу, јер по карактеристикама често личе на обољења изазвана патогеним микроорганизмима или нутритивним дефицитом или дисбалансом. Степен здравствених поремећаја зависи од количине токсина у храни и дужине његовог уношења у организам као и од врсте и категорије животиња.

Присутност микотоксина у храни за животиње је неизбежна па је неопходно тестирање сировина и производа да би храна за људе и животиње била сигурна за употребу. Штете у живинарству и свињарству које настају услед микотоксикоза, услед директних губитака због угињавања животиња или, још чешће, индиректне због пада производних и репродуктивних способности животиња, наметнуле су потребу за континуираним мониторингом хигијенске исправности крмних смеша за исхрану ових животињских врста.

Током петогодишњег периода (2007–2012) анализирана су укупно 104 узорка са територије Републике Србије намењена исхрани свих категорија живине и то смеше за почетни и завршни тов бројера (50 узорака) и за кокоши носиље (54). Анализом је обухваћено и 57 узорака крмних смеша намењених исхрани свих категорија свиња и то смеше за исхрану младих (20 узорака) и старих категорија (37 узорака) као и 196 узорака хранива која се најчешће користе приликом формулесања obroка за наведене животињске врсте (кукуруз, сојина и сунцокретова сачма). За анализу узорака коришћени су метода танкослојне хроматографије и елиса тест. Добијени резултати су поређени са тренутно важећим Правилником о квалитету хране за животиње (Службени Гласник РС 41/09) који се примењује од 1.05.2010. године и где се у делу о максимално дозвољеним количинама штетних материја (члан 99) износе вредности о максимално дозвољеној количини микотоксина у храни за животиње. Број и врста микотоксина варира у односу на врсту смеша, као и у односу на поједине године што се може довести у директну везу са климатским факторима, односно просечном годишњом влажношћу. Чињеница да у испитиваним узорцима није утврђено присуство афлатоксина указује да у нашим условима токсинпродукујуће гљивице не наилазе на погодно тле за продукцију овог токсина, као и на одсуство појединих хранива у производњи потпуних крмних смеша за свиње и живину које представљају традиционалне изворе афлатоксина (кикирикијева уљана сачма). Добијени резултати представљају охрабрујућу чињеницу с обзиром на релативно мали број неисправних смеша и хранива. Међутим, чињеница да се ради о ограниченом броју узорака хране које смо добијали на анализу упућује на опрез и даљи константан мониторинг присуства микотоксина у храни за животиње.

КЉУЧНЕ РЕЧИ: микотоксини, свиње, живина, сточна храна

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