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THE IMPORTANCE OF ANTIOXIDANTS IN THE PROTECTION AGAINST MYCOTOXICOSES IN FARM ANIMALS

ABSTRACT: Mycotoxins are biologically active substances that are synthesized by saprophytic and parasitic fungi, and which, when taken into organism by ingestion, can provoke intoxications known as mycotoxicoses. Farm animals show different susceptibility to mycotoxins depending on various factors: genetic (species and breeds), physiological (age and obesity) and environmental (hygienic and climatic). One of the mechanisms of mycotoxin activities is peroxidation of lipids brought about directly by the production of free radicals or by increased sensitivity of tissue to peroxidation. Peroxidation of lipids provoked by mycotoxins is caused by low level of natural antioxidants, so they have a crucial role in the protection against mycotoxins. Nutritive stress can influence negatively the relationship between antioxidants/pro-oxidants, and mycotoxins are nowadays regarded as leading factors of stress induced by nutrition. This optimal relationship can be regulated by the use of antioxidants in food (selenium, vitamin E, carotenoids, etc.) known to prevent tissue damages caused by free radicals. Selenium and vitamin E are essential nutrients which contribute to the preservation of animal health by realizing mutual biological activities in the organism. This paper presents the findings on mechanisms of the action of different species of mycotoxins and the importance of antioxidative protection in farm animals, as well as the results of our investigations of influence of mycotoxins on the occurrence of some reproductive disorders in pigs.

KEY WORDS: antioxidants, farm animals, mycotoxins

INTRODUCTION

Mycotoxins are secondary products in the metabolism of molds and they represent a global problem in supplying people and animals with food. For a number of years it has been thought that mycotoxins appear only in a small number of feedstuffs. However, today it is known that they are present in almost all feedstuffs. More than 30 mold metabolites are considered toxic for both people and animals whilst 25% of cereals in the world are contaminated by mycotoxins. *Fusarium* species can inhabit the cereals during the period of

their growth and can create "field mycotoxins". *Aspergillus* and *Penicillium* mostly develop after the harvest and their toxins are called "storage mycotoxins". The trade of cereals results in the spread of mycotoxins, so they may also appear in feedstuffs in the regions where it is rather unusual (S u r a i and D v o r s k a , 2005).

A growing presence of mycotoxins is a consequence of significant climatic changes. Extremely high, but also low temperatures, heavy precipitations and drought favor the development of molds and production of their toxic metabolites in animal and human food. Due to their harmful effect on the health of animals and people, mycotoxins are called "silent killers", "invisible thieves" and "natural toxins" (W o o d, 1992). There are more than 300 mycotoxins present in nature, but so far the toxic effect has been identified for only about 30 mycotoxins. Therefore, negative laboratory findings do not indicate the absence of mycotoxins, but the absence of 30 mycotoxins identified so far. As for the rest of mycotoxins, no precise answer can be given. Sampling of material for the analysis of mycotoxins is rather complicated since synergistic action in low concentrations can cause bigger problems than one mycotoxin in greater quantity (S u r a i et al., 2008).

MECHANISMS OF TOXICITY OF MYCOTOXINS

The main mechanisms of toxicity of mycotoxins are: inhibition of the protein, DNA and RNA synthesis, damages of DNA, lipid peroxidation, the change in structure and function of membrane and starting off the programed death of cell. The consequences are: immunosuppression, hepatotoxicity, nephrotoxicity, neurotoxicity and gonadotoxicity (S u r a i and D v o r s k a , 2004). Mycotoxins may inhibit the activity of gluconeogenetic enzymes, the function of mitochondria (inhibition of the enzymes of tricarbonate cycle), they may disturb the functions of nucleic acids and the synthesis of proteins. They demonstrate hepatotoxic and hepatocarcinogenic action through the peroxidation of lipids. They can also have immunomodulatory effect even when they are present in food in concentrations below the limit of detection. They suppress humoral and cellular immunity, change the activity of T and B lymphocytes, decrease the production of antibodies and disturb the function of macrophage.

Not all domestic animals are susceptible to mycotoxins to a same degree. The most susceptible ones are pigs, cattle and poultry, while the least susceptible are sheep and goats. Also, young and quite old animals are the most susceptible. Poor feeding, bad hygiene and the way of keeping may increase the susceptability of animals to mycotoxins. High concentration of mycotoxins in food can even lead to death of a great number of heads in a short time period. However, low contents of mycotoxins are also problematic, due to persistent depression of animals (J o k i ć et al., 2004).

Research by J o k i ć et al. (2003) pointed to the negative effects of some mycotoxins on sows' reproductive characteristics in two different periods. In the first period of examination the animals were fed with silage, wet maize

grain, soya and sunflower grits all having high levels of mycotoxins. In the second period, the animals were fed with diets which did not contain prohibited quantities of mycotoxins (artificially dried maize grain, soya and sunflower grits) (Table 1).

Tab. 1 – Average content of mycotoxins in some fodder

Fodder	Mycotoxins, mg · kg^{-1}			
	Zearalenon	Aflatoxin B_1	Aflatoxin G_1	Ochratoxin
First Period				
Silaged wet corn kernels	3.4	0.08	0.01	3.0
Soybean meal	1.6	-	-	0.1
Sunflower meal	5.32	0.052	-	0.66
Second Period				
Artificially dried corn kernels	0.1	-	-	0.1
Soybean meal	0.07	-	-	-
Sunflower meal	0.2	-	-	0.02

The mean values for the level of mycotoxins in some feeds in the second period were lower than those prescribed by the Book of Regulations on maximum quantities of harmful matters and ingredients in animal feeds. The level of F-2 toxin ranged from 0.07 mg/kg in soya grits to 0.2 mg/kg in sunflower grits. Ochratoxin was present at the level of 0.02 mg/kg in sunflower grits, and 0.1 mg/kg in artificially dried maize grain, but the presence of aflatoxin B_1 and aflatoxin G_1 was not confirmed.

The results of the insemination of sows in the first and second period of trial are shown in Table 2.

Tab. 2 – Results of insemination of sows

Parameter		Period		Difference
		I	II	
Number of attempts		6180	7052	
Delivery Parity		3.95	3.83	
Drying-estrus interval (days)		21.01	15.87	
Success of insemination (conception)	n	4415	5932	12.68^{***}
	%	71.44	84.12	
Failure to impregnate	n	1765	1120	12.68^{***}
	%	28.56	15.88	
Miscarriage	n	86	95	0.04 NS
	%	1.39	1.35	
Deliveries	n	4237	5690	12.13^{***}
	%	68.56	80.69	

The occurrence of oestrus in sows was rather long in both periods of trial. After the weaning, the oestrus was manifested after 15.87 days in sows fed with diets containing lower content of mycotoxins, while in animals fed with mixture of high level of mycotoxins it appeared after 21.01 days. The success

in sows insemination was better in the second period than in the first (84.12% in comparison to 71.44%). Obtained differences were statistically significant ($P<0.001$). The number of services was greater in the first period than in the second, with 28.56% and 15.88%, respectively, expressed in relative values. The percent of farrowed sows was greater in the second period (80.69%) than in the first (68.56%) and these differences were statistically highly significant.

MYCOTOXINS AND APOPTOSIS

Mycotoxins incorporated into the cell membranes may lead to lethal changes. They may provoke the change in the composition of fatty acids and peroxidation of polyunsaturated fatty acids in membrane, leading to a damage of membrane receptors and loss of membrane function due to the change in elasticity and porousness. The maintenance of tissue homoestasis involves removing of spent and damaged cells. This process is a programed apoptosis of cells. It is developed by initiating the death signal in the plasma membrane, creating pro-apoptotic oncoproteins, by activation of proteases and endonucleases. Final result is irreversible process that leads to cell's death. In apoptosis, cells shrink, the nucleus gets smaller, chromatin is condensed, DNA splits into fragments and proteins of kinase are activated.

Reactive oxygen species have an important role in the process of apoptosis, including the onset of the reinforcement of apoptosis. The decrease of GSH level within the cell, that is, its consumption by the cell, may intensify apoptosis. On the contrary, the increase of GSH level decreases this process. Many studies have confirmed that mycotoxins decrease the level of GSH, thus initiating apoptosis. T_2 toxin is a potent apoptosis agent. Trichotecenes induce nucleosomal fragmentation of DNA (N a g a s e et al., 2001). Today the apoptosis is being considered as a mutual mechanism of toxicity of various mycotoxins.

MYCOTOXINS AND ANTIOXIDANTS

Mycotoxins can lead to lipid peroxidation directly, or they can increase the tissue sensitivity to peroxidation. Lipid peroxidation caused by mycotoxins is a consequence of decreased level of natural antioxidants (D v o r s k a and S u r a i, 2001). Antioxidants are of great importance in the protection against mycotoxins (G a l v a n o et al., 2001). Selenium and vitamin E are key ingredients of food and they play the role in antioxidative protection. Vitamin E, as an integral component of lipid membrane, neutralizes free radicals. It represents the first line of cell`s defence against free radicals and it is the keeper of cell's integrity. Selenium plays its role through the enzyme GSH-Px. The level of this enzyme increases in plasma along with the increase of the concentration of selenium in food or water, what is a good indicator of biological adoption of selenium (S a n k a r i , 1985; H a s s a n , 1987; T o d o r o v i ć , 1990; M i h a i l o v i ć et al., 1991). With na increase of selenium level above the

necessary, the activity of GSH-Px shows the effect of plateau so that higher concentrations of selenium do not lead to further increase of the activity of this selenoenzyme (Meyer et al., 1981; Mihailović et al., 1997; Joksimović-Todorović et al. 2005a, b, 2006a, b). Organic selenium demonstrates better antioxidative protection than non-organic, due to better resorption (Jokić et al., 2005), and in combination with vitamine E it demonstrates protection in chicks under stress and/or infected by aflatoxin (Stanley et al., 1998).

Sometimes selenium and vitamin E show synergistic activities and sometimes not. The lack of synergism shows that selenium and vitamin E have different forms of protection against oxidative stress, or that some mechanisms in which peroxidation is not present are also involved in the defence. These mechanisms can function together, but they can also occupy different places depending on situations created (Allison and Laven, 2000).

Protective action of selenium against aflatoxin has been confirmed in mammals as well. Pigs were protected against the action of AFB1 when they received 2.5 mg Se/kg through diet (Davila et al., 1983). Selenium also displays protective effect in toxicosis induced by T-2 and DON mycotoxins. Furthermore, acute lethal toxicity of T-2 toxin decreases with the use of selenium (Yazdanpanah et al., 1997). Not only selenium but also some other antioxidants show protective effect in lipid peroxidation caused by mycotoxins: vitamin A and E, ascorbic acid, coenzyme Q 10, synthetic antioxidants and plant extracts. The use of Mycosorb and organic selenium is highly efficient in preventing peroxidation in the liver of chicks infected by T-2 toxin (Surai, 2002). A simultaneous use of antioxidants and absorbents of mycotoxins (of wide range) today represents the best protection of poultry against mycotoxicoses (Weber et al., 2006).

CONCLUSION

Mycotoxins are today regarded as leading factors of nutritive stress which affect unfavorably the relationship of antioxidants/pro-oxidants. Considering the fact that the antioxidants/pro-oxidants balance in the cell (redox status) is responsible for regulating apoptosis, it is likely that natural antioxidants and selenoproteins (GSH-Px, tireodoxin reductase and methionine sulphoxid reductase B) may be involved in the prevention of apoptosis caused by mycotoxins.

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ЗНАЧАЈ АНТИОКСИДАНАТА У ЗАШТИТИ ОД МИКОТОКСИКОЗА КОД ДОМАЋИХ ЖИВОТИЊА

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Резиме

Микотоксини су биолошки активне материје које синтетишу сапрофитне и паразитске гљивице, а унети ингестијом у организам изазивају тровања која се називају микотоксикозе. Домаће животиње су различито осетљиве на микотоксине зависно од различитих фактора: генетских (врсте и расе), физиолошких (старости и ухрањености) и услова средине (хигијенских и климатских). Један од механизама деловања микотоксина је пероксидација липида директно производњом слободних радикала или повећањем осетљивости ткива на пероксидацију. Пероксидација липида изазвана микотоксинима је проузрокована ниским нивоом природних антиоксиданата, тако да они имају кључну улогу у заштити од микотоксина. Нутритивни стрес неповољно утиче на однос антиоксиданта/про-оксиданта, а микотоксини се данас сматрају водећим факторима стреса изазваних исхраном. Овај оптималан однос може се регулисати употребом антиоксиданата у храни (селен, витамин Е, каротиноиди, и др.) који спречавају ткивна оштећења узрокована слободним радикалима. Селен и витамин Е су есенцијални нутрицијенти који доприносе очувању здравља животиња остваривањем заједничких биолошких активности у организму. У овом раду биће приказана сазнања о механизмима деловања различитих врста микотоксина и значаја антиоксидативне заштите код домаћих животиња, као и резултати наших испитивања утицаја микотоксина на појаву појединих репродуктивних поремећаја код свиња.