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MOULD AND MYCOTOXIN CONTAMINATION OF PIG AND POULTRY FEED*

ABSTRACT: During ten-year period (1995—2004), a totat of 756 analyses of pig and poultry feed was performed. Standard methods were used for microbiological determination. Qualitative and quantitative analyze of mycotoxins was performed by TLC technique.

Feed for young categories contained from 100 to 3,400,000 CFU/g of feed. In 35.71% of all samples the detected amount was above acceptable levels. Feed for adult categories contained from 800 to 8,000,000 CFU/g of feed. In only 7.54% of samples this amount was over the tolerable level. Species determination revealed great heterogeneity, with the most common findings of *Penicillium* spp. (28.38%), *Aspregillus* spp. (26.37%), *Mucor* spp. (24.67%), *Fusarium* spp. (11.33%) and *Rhizopus* spp. (9.22%).

The amount and type of mycotoxin varied depending on the feed category as well as on year of detection, implicating a strong influence of climatic factors and average humidity of the specified year. In a total of 320 analyzed feeds for pigs and poultry the characteristic finding was a combined contamination with two or three mycotoxins.

In 161 samples of feed for young animals the presence of AFB1, F-2 and OTA was detected in 36, 161 and 161 samples, respectively, while in 33, 83 and 71 samples the detected amounts were above tolerable levels.

In 159 samples of feed for adult animals the presence of AFB1, F-2 and OTA was detected in 32, 159 and 159 samples, respectively, while in 31, 65 and 99 samples the detected amounts were above tolerable levels.

KEY WORDS: mould, mycotoxin, feed, pig, poultry

INTRODUCTION

Spoilage of the feed generally means the deviation of standard quality, which incorporates changes of organoleptic properties, nutritional value, as well as of hygienic properties. Spoiled feed could be potentially harmful, but

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not necessarily. Nevertheless, medical aspect of spoiled feed is expressed by the decrease of optimal animal performance, incidence of metabolic disorders, health disturbance, even with lethal outcome. Spoilage of feed is caused by chemical, physical, and biological effects. One of the most common is the presence of microorganisms. Because of their paired effects, via direct impact on nutritive value and/or by the production of mycotoxins, the presence of fungi and moulds in feed is considered as a very important issue in the determination of the overall feed quality. Thus, it could be interesting to evaluate the results of feed used in pig and poultry nutrition, analyzed in our laboratory, and to compare the results with the current legislative, as well as with our earlier findings (Sinovec et al., 1989; Šefer et al., 1989; Nedeljković et al., 1994; Šefer et al., 1994).

MATERIAL AND METHOD

During ten-year period (1995—2004), a total amount of 756 analyses of feed samples for all the categories of pigs and poultry was performed. Standard microbiological methods were used for the determination of microorganism presence. Mycotoxins were detected qualitatively and quantitatively by TLC chromatography (AOAC, 1980). All the results were statistically analyzed and compared with the maximal tolerable amounts stipulated by the current legislative (*Pravilnik*, 1990).

RESULTS

Feed for young categories contained from 100 to 3,400,000 CFU/g of feed. In 35.71% of all samples, the detected number was above maximum tolerable levels. Feed for adult categories contained from 800 to 8,000,000 CFU/g of feed. In only 7.54% of samples this amount was over the tolerable level. Regarding the category of animals, an interesting finding was the fact that feed for young animals is more often contaminated with higher amounts of moulds than allowed (Table 1).

Table 1. Mould contamination of fed for pigs and poultry

	Pigs			Poultry	
	Piglets	Fattening	Reproduction	Broilers	Layers
No. of samples	88	61	40	136	111
Over max. level, %	25	4.91	7.5	42.64	9

Species determination revealed great heterogeneity, with the most common findings of *Penicillium* spp. (28.38%), *Aspregillus* spp. (26.37%), *Mucor* spp. (24.67%), *Fusarium* spp. (11.33%) and *Rhizopus* spp. (9.22%).

The amount and type of mycotoxin varied depending on the feed category as well as on year of detection, implicating a strong influence of climatic

factors and average humidity of the specified year. Out of a total of 320 analyzed feeds for pigs and poultry the characteristic finding was a combined contamination with two (78.75%) or three (21.25%) mycotoxins.

Out of 161 samples of feed for young animals the presence of AFB1, F-2 and OTA was detected in 36, 161 and 161 samples, respectively, while in 33, 83 and 71 samples the detected amounts were above the tolerable levels.

Out of 159 samples of feed for adult animals the presence of AFB1, F-2 and OTA was detected in 32, 159 and 159 samples, respectively, while in 31, 65 and 99 samples the detected amounts were above the tolerable levels (Table 2).

Table 2. Feed samples contaminated with mycotoxins

		No of samples	$\bar{X}$		Sd	Over max. tolerable levels
Piglets	AFB1	23	0.05	±	0.02	13
	F-2	87	5.06	±	2.74	82
	OTA	87	0.27	±	0.23	87
Fattening	AFB1	8	0.06	±	0.04	8
	F-2	35	3.97	±	2.33	30
	OTA	35	0.31	±	0.14	29
Reproduction	AFB1	9	0.06	±	0.04	2
	F-2	36	5.25	±	3.20	34
	OTA	36	0.27	±	0.11	7
Broilers	AFB1	13	0.05	±	0.04	9
	F-2	74	5.14	±	2.69	0
	OTA	74	0.26	±	0.15	70
Layers	AFB1	16	0.04	±	0.03	16
	F-2	88	5.28	±	2.61	0
	OTA	88	0.23	±	0.12	41

DISCUSSION

Fungi and mould growth in feedstuffs is associated with the utilization of nutrients from the host, causing alteration in the nutritional content of the feedstuff. The germ of the grain is the main site for *Aspergillus* spp. development, thus the reduction of energy value due to fat utilization in contaminated grain should be expected. Having in mind that young animals are usually fed with high-energy feed, extra supplementation of oil due to decreased energy value will increase feed cost. On the other hand, formulating feed upon ingredient tables and not taking into account nutritional damage of specific loads could lead to undernutrition and decrease of animal performance. The consumption of mouldy feed caused growth depression in chicks (F r i t z et al., 1973). Not only fat, but also sugars are exposed to mould utilization, leading

to further decrease of nutritive value. The reduction of fat and carbon hydrates content due to mould growth and respiration caused a decrease of 5% in metabolical energy level (Bartov, 1982).

The changes in protein and amino acids content of mouldy grains are not well correlated, as it is the case with energy sources. Relative protein content in mouldy grains was slightly higher than in mould free ones (Cook, 1994), but probably due to a more intensive utilization of fat and carbohydrates compared to protein. Nevertheless, the total nitrogen content in moldy grains was decreased. Regarding the amino acids, it was concluded that the changes occurred in the amounts of some, especially essential ones. After moulding, a decrease in lysine (45%), arginine (50—54%), histidine (49.5%) and cysteine (74.5%), as well as an increase in methionine (34.5—54%) was observed (Kao and Robinson, 1972).

The development of moulds on stored feedstuffs may result from contamination in the field or during the storage. Isolated species in our case are mostly storage contaminators, implicating that the high number of contaminated feed is most probably the result of manipulative mistakes during storage of feedstuffs or feed. Inadequate environmental conditions in storage facilities, lack of control and other factors during feed production and manipulation could strongly contribute to the aggravation of this problem.

Adequate judgment of detected number of microorganisms in feed is even more demanding due to ambiguous legislative statements regarding tolerable amounts, especially when young and adult categories are in question. More precise criteria are needed concerning exact definitions of age and productive specificities of different animal species.

During unfavourable environmental conditions moulds are able to change metabolical pathways, which is consider to be a defensive mechanism. Secondary toxic metabolites, also known as mycotoxins, are produced by several genuses of moulds. The estimation is that approximately 25% of global grain production is contaminated by known mycotoxins (Davegowda et al., 1998), while even greater percentage could be contaminated with still unknown ones. Regarding those facts, the presence of mycotoxins in animal feed represents a great problem for animal production in our country. During 1999—2000 the presence of zearalenone, ochratoxin A, aflatoxin B_1 and T-2 toxin was found out in 72.3—74.5, 41.2—63.6, 20.1—21.65 and 29.7—45.1% of scrutinized feed samples (Bočarov-Stančić i sar., 2000). Moreover, laboratory data demonstrate that over 70% of feed samples are contaminated with two or more mycotoxins in the amounts above maximal approved limits (Šefer et al. 1994; Mašić et al., 2002).

Some of them have great nutritional, medical and economical importance in animal production (aflatoxins, ochratoxins, zearalenone, trichothecenes). They are mostly produced by *Aspregillus, Penicillium* and *Fusarium* species. Their impact on animal's health and performance are relatively well described in the literature. Functional and structural changes in target tissues, organs and systems (Humphreys, 1988; Willie and Morehouse, 1978) lead to deterioration of health with possible death, decrease in performance and significant economic losses.

Special attention should be paid to the impact of mycotoxins in human consumers. Of greatest concern in humans (Ellis et al., 1991) is AFB_1 implicated role in primary liver cancer in some geographical areas in Africa and Asia, where values of high dietary AFB_1 daily intake were encountered. It is difficult, however, to establish a possible casual role of AFB_1 in geographical areas where the incidence rate of primary liver cancer is very low, as in Europe and North America, and where the dietary AFB_1 daily intake is very low.

OTA is the main cause of an irreversible and fatal kidney disease (Balkan endemic nephropathy). It could be the result of the consumption of commodities directly contaminated with toxigenic strains of fungi, as well as by the consumption of meat of animals which have eaten OTA-contaminated feeds (Blunden et al., 1991).

F-2 toxin can produce oestrogenisation and pseudogravidity in women and it is related with prostate carcinoma. On the other hand, zearalenone derivates used as chemotherapeutics can benefit in menopause disturbances. Commercial application exists in some countries where α-zearalenone is used as growth stimulator in steer and lamb breeding (Hidy et al., 1977, US Food and Drug Administration 1980). Significance of zearalenone is connected with climate conditions in Balkan region that are optimal for *Fusarium* growth, as well as for the F-2 production. According to recent investigation zearalenone is the mycotoxin with the highest prevalence in feed for swine, with an increasing trend (Mašić et al., 2002), indicating that more than 75% of positive feed samples contained F-2 above the maximal tolerable levels.

CONCLUSION

The changes in the nutritive value that can occur after mould contamination of feed should be taken into consideration when feed efficiency is estimated regarding animal's performance. Although the major role in detrimental effects is contributed to the mycotoxins, it would be much precise if both factors — decrease of nutritive value and toxin impact — could be regarded together. The most important role of this interaction could be observed when low levels of mycotoxin are present.

Prevention and control of mould development should be one of the major tasks in the efforts to provide safe and adequate feed for domestic animals. Permanent monitoring is needed on all levels of production and storage, as well as the use of known methods to reduce mould contamination or toxin content in feedstuffs and feed. Improved legislative could further positively contribute to the better control and solution of mouldy feed problems. Preserved feed quality is the main condition in assurance of expected animal performance and good health, leading further to high quality of animal products, safe for human nutrition.

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КОНТАМИНАЦИЈА СМЕША ЗА ИСХРАНУ СВИЊА И ЖИВИНЕ ПЛЕСНИМА И МИКОТОКСИНИМА

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Резиме

Током десетогодишњег периода (1995—2004) извршено је укупно 756 анализа узорака сточне хране намењених за исхрану живине и свиња. За микробиолошку анализу узорака коришћене су стандардне методе, а квалитативно и квантитативно испитивање наведених микотоксина извршено је ТЛЦ методом.

Смеше за младе животиње садржале су од 100 до 3.400.000 плесни/gr, при чему је чак 35.71% испитиваних узорака садржало недозвољен број плесни. Смеше за одрасле животиње садржале су од 800 до 8.000.000 плесни/gr, при чему је свега 7.54% испитиваних узорака садржало недозвољен број плесни. Врсте изолованих родова плесни показују велику хетерогеност, а најчешће су детектоване *Penicilium* spp. (28.38%), *Aspergillus* spp. (26.37%), *Mucor* spp. (24.67%), *Fusarium* spp. (11.33%) и *Rhisopus* spp. (9.22%).

Количина и врста микотоксина варира у односу на врсту смеше, као и у односу на поједине године, што може да се доведе у директну везу са климатским факторима, односно са годишњом влажношћу. Испитано је укупно 320 смеша за исхрану свиња и живине, а карактеристично је да је велик број смеша био контаминиран са два, односно три микотоксина.

Од укупно 161 узорка хране за младе животиње присуство АФБ1, Ф-2 и ОТА утврђено је у 36, 161 и 161 узорку, од чега је, истим редом, 33, 83 и 71 узорак садржао наведене микотоксине изнад дозвољене границе.

Од укупно 159 узорака хране за одрасле животиње присуство АФБ1, Ф-2 и ОТА утврђено је у 32, 159 и 159 узорака, од чега је, истим редом, 31, 65 и 99 узорака садржало наведене микотоксине изнад дозвољене границе.