CONCENTRATION OF MYCOTOXINS IN FORAGE UNDER PROBLEMATIC CASES

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Summary. Contamination of animal forage with mycotoxins in Lithuania have been evaluated only during the last decade. There is no information about the distribution of mycotoxins from earlier years. Veterinary doctors, and especially pig breeders often observe clinical and pathologic cases that are typical to mycotoxicoses. Under these problematical cases, samples of animal forage were evaluated with VICAM fluorimeter. It has been thought, that in Lithuania fusariotoxicoses causes the most of the problem, however other mycotoxins (ochratoxins, aflatoxins) are detected in forage as well. Aflatoxins are most frequently detected in imported foodstuffs.
Keywords: mycotoxins, DON, Zearalenone, T-2 toxin, ochratoxin, aflatoxin, VICAM

MIKOTOKSINŲ KONCENTRACIJA PAŠARUOSE PROBLEMINIAIS ATVEJAIS

Santrauka. Mikotoksinai Lietuvoje naudojamuose pašaruose detaliai tyrinėti tik paskutinį dešimtmetį. Iki tol neturėta duomenų apie jų paplitimą ir kiekius. Veterinarijos gydytojai, o ypač kiaulių augintojai, neretai aprašo mikotoksikozėm būdingus klinikinius ir patologinius pakitimus. Todėl, pasireiškus tokiems probleminiams atvejams, buvo tiriami pašarai amerikiečių gamybos VICAM fluorimetru. Kaip ir buvo manyta Lietuvoje labiausiai aktuali fuzariotoksikozių problema. Tačiau randami ir kiti mikotoksinai (ochratoksinai, aflatoksinai). Beje aflatoksinai dažniau aptinkami importinėse pašarinėse žaliavose. Iš fuzariotoksinų didžiausi kiekiai pašaruose aptinkami zearalenono ir deoksinivalenolo.
Raktažodžiai : mikotoksinai, DON, zearalenonas, T–2 toksinas, ochratoksinas, aflatoksinas, VICAM.

Introduction. Mycotoxicology, as a subject and the spread of the mycotoxins became of great interest in 1960, when the cause of massive death of ducklings in the United Kingdom had been established. In 1992 mycotoxicologic contamination of forage was evaluated in several European countries – Austria, Denmark, France, Germany, Hungary and Sweden (Bauer J.,1993). According to the results obtained, fusariotoxins (DON and zearalenone) are the most widely spread in the forage of Central European countries. It is of interest to note, that the incidence of spread of DON in forage, produced in Finland, Germany and Austria exceeds 90% (Hintika El. and others, 1988). Concentration of these mycotoxins varies form year to year and depends on the growth conditions of forage. In 1991 the incidence of these mycotoxins decreased. DON in Austria was determined in 55% of the cases and in Sweden – in 35% (Petterson H. and others, 1992). Climatic conditions in Lithuania are similar to these in southern Sweden and therefore suitable for mycotoxin production.

Zearalenone is observed in nearly all agricultural products, raw materials, or mixtures (Drochner W., 1998). High concentrations of this and other fusariotoxins are found in various countries of Middle Europe (Austria, Germany, France, countries of former Yugoslavia) (Drochner W. and others, 2001; Brake and others, 2000). The average quantities of zearalenone depending on each year varies from 0.002 to 0.3 mg/kg.

Ochratoxin is more frequently and in greater quantities determined in wider spread in northern European countries (Denmark, Poland). It is found in barley which upon ingestion gives clinical signs of porcine nephropathy. It has been observed in Austria that ochratoxin contamination of cereals was found on the average in 44% of the cases (Krogh P. and others, 1995). In Denmark, during the 1989-1991 year period ochratoxin was determined in 30-50 % of the cases evaluated, and contamination of wheat products in Sweden was observed in 22 to 36% of the cases (Breiholtz A. and others, 1991).

Aflatoxins are more frequently found in products imported from developing countries. Contamination of forage in Europe by aflatoxins is not very pronounced. Most frequently these toxins are observed in imported soy beans and peanuts. For this reason nearly all countries in Europe have established maximum allowed concentrations of this toxin in forage (Neal S.E. and others, 1992).

The aim of the present work was to analyze concentrations of various mycotoxins (aflatoxins, ochratoxins and zearalenone) in forage, their dynamics during the 3 year period (1997-1999), that caused mycotoxicose-specific symptoms in animals.

Materials and methods. Analysis of the mycotoxins (aflatoxins, ochratoxins, zearalenone) in the forage was carried out in problematic cases, when animals showed clinical signs specific to the intoxication with mycotoxins.

Mycotoxins were quantified during the 1997/1999 year period using VICAM detector. The principle of the detection is measurement of the fluorescence of the aforementioned toxins (aflatoxins, ochratoxins and zearalenone). Various solvents are used to extract mycotoxins from the samples, the extract is then purified through the VICAM immunoadsorbtion columns. Fluorescence is measured in purified and concentrated extract, and based on the fluorescence output, toxin concentration in the sample is determined. Samples were taken according to the general rules

for taking samples of forage materials. Samples consisted of cereals raised in Lithuania and combined concentrates that had some constituents of the imported materials.

Results. The results from the studies are presented in Table 1 and Figures 1, 2 and 3. As it is seen in Table 1, concentration of the aflatoxins decreased in cereals as well as in combined forage that contained imported raw materials. This can be explained by strict control in EU, and other countries.

Table 1**. Distribution of various mycotoxins in forage during the three year period**

Year	Target for evaluation	Aflatoxin, ppm		Ochratoksinas, ppm		Zearalenonas, ppm	
		Samples evaluated	Mean observed concentrations	Samples evaluated	Mean observed concentrations	Samples evaluated	Mean observed concentrations
1997	Cereals	6	0.0054± 0.0017	10	0.010± 0.0037	4	0.30± 0.084
	Combined fodder	17	0.0088± 0.0019	29	0.022± 0.0029	9	0.29± 0.063
1998	Cereals	12	0.0054± 0.0015	10	0.021± 0.0066	17	0.45± 0.053
	Combined fodder	23	0.0069± 0.0021	30	0.029± 0.0041	39	0.47± 0.045
1999	Cereals	4	0.0041± 0.0017	4	0.015± 0.0011	5	0.32± 0.097
	Combined fodder	17	0.0027± 0.00076	15	0.022± 0.0016	19	0.30± 0.033

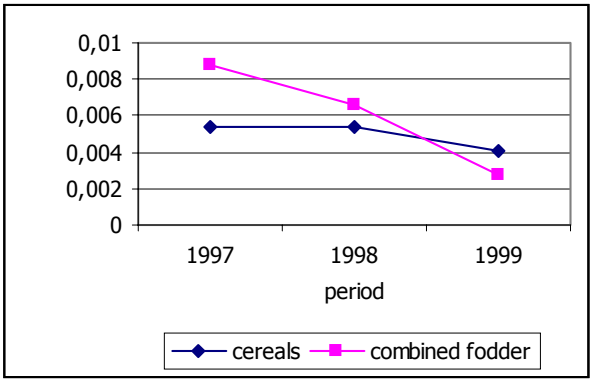

Figure 1. **Distribution of variation in aflatoxin inthree year period**

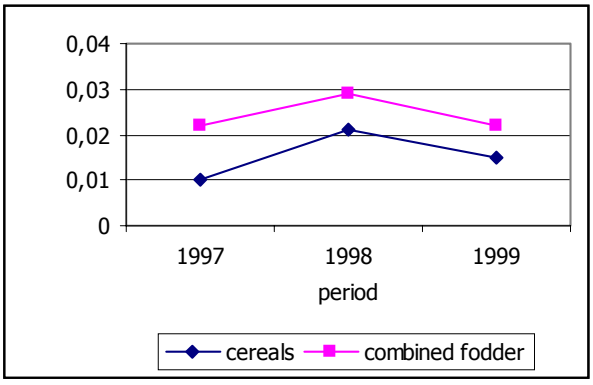

Figure 2. **Distribution of variation in ochratoxin concentration in three year period**

Concentration of aflatoxin in the cereals produced in Lithuania, on average in 1997-1998 was 0.0054 ppm, and in 1999- 0.0041 ppm, however this decrease was not statistically significant (p>0.05). Statistically significant change in aflatoxin concentration in combined fodder was observed between year 1997 and 1999. The decrease was 71%. Major impact on this decrease might have been due to a very strict veterinary control of the imported raw materials.

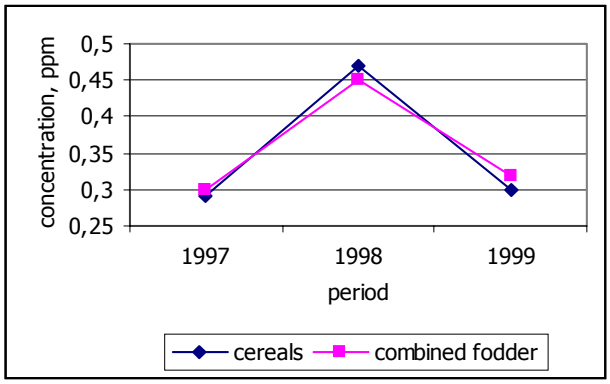

Figure 3. **Distribution of variation in zearalenone in three year period**

Ochratoxin concentration during the period of 1997-1999 varied a little, but the differences were not statistically significant (p>0.05). In the cereals, harvested in Lithuania, ochratoxin concentration was on the average 0.01 ppm in 1997, and increased to 0.021ppm in 1998, with subsequent decrease to 0.015 ppm in 1999. The same tendency was observed in combined fodder. Ochratoxin concentration in combined fodder in 1997 was on average 0,022 ppm, in 1998 increased to 0.029 ppm, and decreased to the initial values - 0.022 ppm in 1999. Higher ochratoxin concentration in combined fodder, compared to that in cereals shows that import of raw fodder materials to Lithuania is not adequately controlled.

There was no significant differences between the concentrations of zearalenone in the cereals, harvested in

Lithuania and in combined fodder. Concentrations increased from 3.3% in 1997 to 4.4% in 1998 and remained the same during the year 1999. This indicates that zearalenone is acute problem not only in Lithuania, but also around the world. For this reason imported raw materials increased zearalenone concentration in combined forage not significantly. The concentration of zearalenone differed between the years, however the differences were not statistically significant ($p>0.05$). On the average, zearalenone concentration in problematic cases in cereals in 1997 averaged 0.30 ppm, and increased to 0.45 ppm in 1998. This can be explained that year 1998 was favorable for fusariozes due to climatic conditions. In 1999 concentrations of zearalenon in cereals averaged 0.32 ppm. Concentration of zearalenone in combined forage amounted 0.29 ppm, 0.47 ppm and 0.30 ppm in years 1997, 1998 and 1999 respectively ($p>0.05$).

Conclusions.1. Aflatoxin concentration in the cereals foraged in Lithuania , does not surpass the allowed concentrations and varies from 0.0011 to 0.0054 ppm. In combined fodder aflatoxin concentration decreased steadily from 0.0088 to 0.0027 ppm ($p<0.01$) through the 1997-1999 year period. Major impact of this decrease might have been due to a very strict veterinary control of the imported raw materials.

2. Fusariotoxicoses cause major problem in Lithuania. Significant amounts of zearalenone (0.47 ppm) are detected in forage. This exceeds the maximum allowed concentration in the forage in Lithuania.

3. Increased levels of ochratoxins (0.02 ppm) have been detected in cereals, harvested in Lithuania as well as in combined forage that contains imported raw materials.

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