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COMBINED INCIDENCE OF MYCOTOXINS IN THE FEEDS GIVEN TO DAIRY COWS

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Abstract: The investigation examined mycological the feeds given to dairy cows in family farms; with the view to determine the level of contamination with some mycotoxins and the incidence of combinations of mycotoxins in the analysed feeds.

A total of 21 feed samples were collected and analysed: 6 samples of a mixture of concentrated feeds and 15 samples of fibrous feeds; from an association of family dairy farms in Giurgiu County.

The mycotoxicological analysis was done with the immunoenzymatic test ELISA to determine: AF; OTA; DON; ZEA and T-2.

The analytical evidence showed that no sample exceeded the allowed limits for aflatoxin; ochratoxin A and DON; while some of the samples exceeded the allowed levels for zearalenone and T-2.

Of the 21 analysed samples; 42.85% (9 samples) displayed combinations of 2 mycotoxins; 23.80% (5 samples) had a single mycotoxin or combinations of 3 mycotoxins; while 4.76% (1 sample) had combinations of 4 and 5 mycotoxins.

INTRODUCTION

Of the total 300-400 compounds acknowledged as mycotoxins; less than 10 have been intensely studied in terms of natural occurrence and toxicity for humans and animals (2). Mycotoxin are metabolised by fungi through a process of secondary metabolism which differs from the primary metabolism by its random character; diversity of formed compounds and specificity of the involved strains. The metabolic chains involved in mycotoxin production are not implied in cell growth; but they respond to signals received by the fungus from the environment. Unlike the primary metabolism which is common to all fungal species; the secondary metabolism is characteristic to one fungal species or even strain due to its genetic particularities. These characteristics determine in their natural environment an unpredictable incidence of the mycotoxins in the feeds and combinations of them are often observed; even though the producing fungi are different.

In the warm climate areas the feeds are contaminated with AF; OT and FP; while in the areas with cool; moist climate the incidence of DON; ZEA; OTA; T-2 and AF is increased.

The 1998 review of Pittet (7) on worldwide reports on the contamination of 27853 samples of raw materials used in human and animal feeding showed that DON and FB are the most widespread mycotoxins. The report also shows that almost 32% of the tested cereal samples were contaminated with ZEA in high concentration (21.5 mg ZEA/kg). Variations in

feed contamination with mycotoxins were recorded both between years and sample reports from the same country.

In many EU member states; due to market globalization after accession; investigation and monitoring programs are developed to evaluate the presence of toxinogenous fungi and of the mycotoxins produced by them (3; 5; 8).

MATERIAL AND METHOD

A total of 21 feed samples were collected and analysed in 2004: 6 samples of a mixture of concentrated feeds (cereal grains; wheat bran and corn bran; sunflower meal; peas; soy) and 15 samples of fibrous feeds; from an association of family dairy farms in Giurgiu County.

The feed samples were collected; processed and analysed according to CE Regulation 401/2006. The mycotoxicological analysis was done by ELISA to determine AF; OTA; DON; ZEA and T-2. The results were processed statistically by JMP6.0 software (SAS Company; 2005).

Results were interpreted according to *CE Regulations No. 1881/2006 and No. 1126/2007* on the limits of mycotoxins in feeds and foods.

RESULTS AND DISCUSSIONS

Table 1 shows the results obtained for the samples of concentrated feeds and fibrous feeds from family dairy farms.

Table 1

The incidence of the mycotoxins AF; OTA; DON; ZEA and T-2 in the 21 fodder assays that were taken from Giurgiu county

No.	Samples feeds	AF ppb	OTA ppb	ZEA ppb	DON ppm	T-2 ppb
1	Fibrous feeds	ND	0.49	ND	0.065	ND
2	Fibrous feeds	ND	ND	120;4	0.081	ND
3	Fibrous feeds	ND	ND	321.7	ND	211.9
4	Fibrous feeds	ND	1.79	119.4	ND	213.3
5	Fibrous feeds	ND	1.19	ND	ND	ND
6	Fibrous feeds	ND	2.62	ND	ND	ND
Limits of variation		ND	0;49 – 2;62	120;4–321;7	0;065–0;081	211;9–213;3
Positive sample no./ %		0 0;0	4 66;6	3 50;0	2 33;3	2 33;3
Negative sample no./ %		6 100	2 33;3	3 50;0	4 66;6	4 66;6
1	Concentrated feeds	0.95	4.22	ND	ND	268.3
2	Concentrated feeds	1.72	1.19	ND	0.017	221.1
3	Concentrated feeds	1.50	2.24	ND	ND	ND
4	Concentrated feeds	1.70	3.19	ND	0.656	ND
5	Concentrated feeds	ND	1.05	227.3	ND	ND
6	Concentrated feeds	ND	ND	ND	0.255	ND
7	Concentrated feeds	1.63	ND	191.8	ND	200.7
8	Concentrated feeds	2.30	ND	ND	ND	287.3
9	Concentrated feeds	0.71	3.21	68.9	0.102	319.7
10	Concentrated feeds	ND	ND	174.5	ND	ND
11	Concentrated feeds	0.74	2.05	ND	ND	ND
12	Concentrated feeds	ND	ND	ND	0.593	268.3

13	Concentrated feeds	ND		2.35		ND		ND		ND	
14	Concentrated feeds	1.56		4.27		ND		0.590		ND	
15	Concentrated feeds	ND		4.96		ND		0.041		ND	
Limits of variation		0;71-2;30		1;05–4;96		68;9–227;3		0;017–0;656		200;7–319;7	
Positive sample no./ %		9	60;0	10	66;6	4	26;6	7	46;6	6	40;0
Negative sample no./ %		6	40;0	5	33;3	11	73;3	8	53;3	9	60;0
Total positive sample no./ %		9	42;8	14	66;6	7	33;3	9	42;8	8	38;0
Total general negative sample no./ %		12	57;1	7	33;3	14	66;6	12	57;1	13	61;9
References limits		4		5		100		1;25-1;75		< 100	
Sample under references limit total no./%		0	0;0	0	0;0	6	28;57	0	0;0	8	38;09

*ND-not determined

Table 1 shows that in the samples of fibrous feeds AF was determined in proportion of 0% (0 samples); OTA was determined in proportion of 66.6% (4 samples); ZEA in proportion of 50.0% (3 samples); DON in proportion of 33.3% (2 samples) and T-2 in proportion of 33.3% (2 samples); in the samples of concentrated feeds: AF was determined in proportion of 60.0% (9 samples); OTA in proportion of 66.6% (10 samples); ZEA in proportion of 26.6% (4 samples); DON in proportion of 46.6% (7 samples) and T-2 in proportion of 40% (6 samples).

The analysis of the 21 samples from Giurgiu County show that:

- AF was not determined in 57.1% samples (12 feed samples: 6 samples fibrous feeds and 6 samples of concentrate mixtures) and was determined in 42.8% samples (9 samples of concentrate mixtures) with values ranging between 0.71 and 2.30 ppb. None of these values exceeded the allowed limit (4 ppb).

- OTA was not determined in 33.3% samples (7 feed samples: 2 samples fibrous feeds and 5 samples of concentrate mixtures) and was determined in 66.6% samples (14 feed samples of which 4 samples fibrous feeds and 10 samples of concentrate mixtures) with values ranging between 0.49 and 4.96 ppb. The values were below the admitted limit (5 ppb); in only one sample the value was very close to the admitted value (4.96 ppb).

- ZEA was not determined in 66.6% samples (14 feed samples: 5 samples fibrous feeds and 11 samples of concentrate mixtures) and was determined in 23.8% samples (5 feed samples of which 1samples fibrous feeds and 4 samples of concentrate mixtures) with values ranging between 68.9 and 227.3 ppb. AT the time of the analysis only one sample of fibrous feed was above the admitted limit (300 ppb) with the value of 321.7 ppb; while presently 5 of the 6 samples in which ZEA was determined are in excess of the admitted limit (100 ppb).

- DON was not determined in 57.1% samples (12 feed samples: 8 samples fibrous feeds and 4 samples of concentrate mixtures) and was determined in 42.8% samples (9 feed samples of which 2 samples fibrous feeds and 7 samples of concentrate mixtures) with values ranging between 0.017 and 0.656 ppm. None of these values exceeded the allowed limit (1.25-1.75 ppm).

- T-2 was not determined in 61.9% samples (13 feed samples: 4 samples fibrous feeds and 9 samples of concentrate mixtures) and was determined in 38.0% samples (8 feed samples of which 2 samples fibrous feeds and 6 samples of concentrate mixtures) with values ranging between 200.7 and 319.7 ppb. All of these values exceeded the allowed limit (100 ppb).

By category of mycotoxin; in decreasing order; the upper admitted limit was exceeded by 38.09% for T-2 (8 samples); by 28.57% for ZEA (6 samples) and by 0.0% for AF; OTA and DON (0 samples).

The statistical analysis showed that there were no significant differences between the concentrate mixtures and the fibrous feeds concerning the level of contamination with OTA (p = 0.089); significant differences between the two categories of feeds (p = 0.531) were observed for the contamination with ZEA; no significant differences between the two categories of feeds (p = 0.059) were observed for the contamination with DON; the two categories of feeds differ at the limit of significance (p = 0.041) for the contamination with T-2.

The data from Table 1 show that:

- In Giurgiu County; of the 21 analysed samples; the identified mycotoxins in decreasing order were: OTA in a proportion of 66.6% (14 samples); AF; ZEA and DON in a proportion of 42.8% (9 samples) and T-2 in a proportion of 38.0% (8 samples).

By category of feeds; in decreasing order; the mycotoxins determined in the fibrous feed were: OTA in a proportion of 66.6% (4 samples); ZEA; DON and T-2 in a proportion of 33.3% (2 samples) and AF in a proportion of 0% (0 samples); in the concentrated feed: OTA in a proportion of 66.6% (10 samples); AF in a proportion of 60.0% (9 samples); ZEA and DON in a proportion of 46.6% each (7 samples) and-2 in a proportion of 40% (6 samples).

Table 2 shows the number; structure and percentage of the combinations of the 5 investigated mycotoxins: AF; OTA; ZEA; DON and T-2 in the 21 analysed samples.

Table 2

The number; structure and percentage of the combinations of mycotoxins AF; OTA; ZEA; DON and T-2 in the 105 analyzed assays of fodder in the year 2004

Number; structure and percentage of the combinations of the mycotoxins no./%														
1 mycotoxin			2 mycotoxins			3 mycotoxins			4 mycotoxins			5 mycotoxins		
Specification	no.	%	Specification	no.	%	Specification	no.	%	Specification	no.	%	Specification	no.	%
OTA	3	14; 28	OTA + DON	2	9;52	AF+ZEA + T-2	1	4;76	AF+OTA+DON+ T-2	1	4;76	AF+OTA+ZEA + DON+T-2	1	4;76
DON	1	4;76	DON+ T-2	1	4;76	AF+OTA + DON	2	9;52						
ZEA	1	4;76	ZEA + DON	1	4;76	OTA+ ZEA+T-2	1	4;76						
			ZEA+T-2	1	4;76	AF+OTA + T-2	1	4;76						
			AF+OTA	2	9;52									
			OTA+ ZEA	1	4;76									
			AF+T-2	1	4;76									
Sample /total sample	5/21	23; 80	Total sample	9/21	42; 85	Sample/total sample	5/21	23; 80	Sample/ total sample	1/21	4;76	Sample/ total sample	1/21	4;76

Table 2 data analysis shows that from the total of 21 analysed samples:

- 42.85% (9 samples) had combinations of 2 mycotoxins; 9.52% (2 samples) of which were OTA+DON and AF+OTA; while 4.76% (1 sample) were DON+T-2; ZEA+DON; ZEA+T-2; OTA+ZEA and AF+T-2; of these combinations; ZEA+DON and AF+T-2 are known for the their synergic action.

- 23.80% (5 samples) had only one mycotoxin; of which 14.28% (3 samples) had OTA şi 4.76% (1 sample) had DON and ZEA or combinations of 3 mycotoxins; of which 9.52% (2 samples) were AF+OTA+DON and 4.76% (1 sample) were AF+ZEA+T-2; OTA+ZEA+T-2 and AF+OTA+T-2;

- 4.76% (1 sample) had combinations of 4 mycotoxins: AF+OTA+DON+T-2 and of 5 mycotoxins; AF+OTA+ZEA+DON+T-2.

Data analysis shows an increased incidence (in excess of 9.52%) of ochratoxin; of the combinations of 2 mycotoxins; OTA+DON and OTA+AF and of the combination of 3 mycotoxins; AF+OTA+DON.

When the mycotoxins have a similar structure and originate from the same species or genus; their manner of action and profile of toxicity is similar (9). Mycotoxins AF and OTA or AF and T-2 ingested simultaneously determine synergic effects in the broilers; both in the liver and kidneys (6).

OTA in association with T-2; was proved to impact the health and productive performance of pigs (lower body weight and lower liver weight; immunosuppression; abnormal blood parameters) (4). OTA and DON may have additive effects.

An experimental research on yeasts cultures has shown that low amounts of DON and ZEA didn't have adverse synergic effect on yeasts growth. Other experiments reported synergic or antagonist effect of the mycotoxins depending on their amount in the experimental mixture. A synergic effect of the mycotoxins produced by *Fusarium sp.;* DON; ZEA and FB was noticed only upon the use of high amounts of the mycotoxins (1).

CONCLUSION

- In Giurgiu County; the mycotoxins identified in the fibrous feeds samples; in decreasing order; were: OTA in a proportion of 66.6%; ZEA; DON and T-2 in a proportion of 33.3% and the absence of AF; in the concentrate feeds samples: OTA in a proportion of 66.6%; AF in a proportion of 60%; ZEA and DON in a proportion of 46.6% and T-2 in a proportion of 40%.
- In the feed samples from Giurgiu County; AF ranged between 0.71 and 2.30 ppb; none of the values exceeding the upper limit (4 ppb); OTA ranged between 0.49 and 4.96 ppb; values below the admitted limit (5 ppb); ZEA ranged between 68.9 and 227.3 ppb; in 5 of the 6 samples exceeding the admitted limit (100 ppb). DON ranged between 0.017 and 0.656 ppm; none of which exceeded the admitted limit (1.25-1.75 ppm). Toxin T-2 ranged between 200.7 and 319.7 ppb; which exceeded the admitted limit (100 ppb).
- The statistical analysis of the results for the samples collected from Giurgiu County shows that there were no significant differences between the two categories of feeds concerning the amount of OTA ($p= 0.089$) and DON ($p = 0.059$). The two types of feed differed at the limit of significance ($p=0.041$) for the amount of T-2;
- The analytical evidences concerning the 21 analysed samples showed that 42.85% (9 samples) had a combination of 2 mycotoxins; 23.80% (5 samples) had only one mycotoxin or combinations of 3 mycotoxins; and 4.76% (1 sample) had combinations of 4 and 5 mycotoxins; respectively.

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