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RESULTS OF ONE-YEAR INVESTIGATIONS OF THE CONTAMINATION OF DAIRY CATTLE FEED AND RAW MILK WITH MOULDS AND MYCOTOXINS

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Abstract: The contamination of 98 dairy cattle feed samples with moulds and mycotoxins (AB1, OTA, ZEA) was investigated. The contamination of 80 raw milk samples with aflatoxin M1 was also tested. Determination of total viable counts per 1g of feed was performed using Koch's standard method. The content of AB1, OTA and ZEA in feed, as well as aflatoxin M1 in raw milk samples, was determined by VICAM fluorimetric method. Results showed that 91% of all tested samples was contaminated with moulds, and total viable mould count per gram ranged from 10 (corn silage – spring) to 4.9 x 10^6 per gram (dried alfalfa – winter). The isolated moulds belong to 20 genera and 72 species. Results of mycotoxicological investigations showed that 42 samples of total 98 tested, were contaminated with mycotoxins. The most frequent was OTA (24 samples, 20.00 - 210.00 μg/kg) followed with ZEA (12 samples, 250.00 – 980.00 μg/kg) and AB1 (3 samples, traces – 18.50 μg/kg). Aflatoxin M1 in raw milk samples wasn't found.

Key words: feed, milk, moulds, mycotoxins

Introduction

Fungi are world-wide distributed microorganisms which can exist in all climate regions. Fungal contamination of feed and food may present a major animal and human health risks throughout the world. The risk reflects in the ability of numerous fungal species to produce harmful metabolites – mycotoxins *(Škrinjar et al., 2009)*. Mycotoxins are common contaminants in the feed chain and they are often suspected to be responsible of animal health damage or death *(Griessler et al., 2009; Tangni et al., 2009)*. Furthermore, mycotoxin ingestion by humans occurs mainly through plant-based foods while the residues and metabolites are present in animal-derived food, and can lead to various health disorders *(Sweeney and Dobson, 1998)*. Considering the importance and diversity of their toxic effects

– carcinogenic, immunotoxic, teratotoxic, neurotoxic, nephrotoxic and hepatotoxic – the occurence of mycotoxigenic moulds in foods and feeds is potentially dangerous for public health and also constitutes a major economic problem *(Dalié et al., 2010)*.

In dairy cattle breeding, various plant commodities are used as feed and could be potentially contaminated by toxigenic moulds and their mycotoxins *(Lanier et al., 2009)*. As it is almost impossible to totally avoid mycotoxins with proper prevention methods, monitoring mycotoxin-producing moulds and mycotoxins in feed is indispensable.

The aim of this study was to determine mould and mycotoxin contamination of different feed samples, used for dairy cattle feeding in four farms in Vojvodina Province of Serbia, covering all seasons in one research year.

Materials and Methods

Contamination of dairy cattle feed samples with moulds and mycotoxins (aflatoxin B1 – AB1, ochratoxin A – OTA, zearalenone – ZEA) was investigated during one reseach year (in all seasons). Raw milk samples were analysed for the presence of aflatoxin M1 (AM1). All tested samples originated from 4 dairy cattle farms from the Vojvodina Province, Serbia.

Total of 98 samples of cattle feed (concentrate – 17, fresh alfalfa – 3, dried alfalfa – 16, hay – 16, corn silage – 18, fresh corn stover - 2, pelleted sugar beet pulp - 14, fresh sugar beet pulp – 3, fresh beet leaf – 1, fresh beet – 1 pelleted malt spent grains – 6, sunflower meal – 1) and 80 samples of raw milk (5 samples from each of four farms sampled in each seasaon during the research year) were used in this investigation.

Mycological investigation. Determination of total viable counts per 1g of feed was performed using Koch's standard method. Sabouraud maltose agar (SMA) with streptomycin (0.01 – 0.02%) was used as an isolation medium. Incubation was carried out at 25 °C for 7 to 10 days. Identification of fungal species was done according to *Ellis (1971), Pidopličko and Miljko (1971), Nelson et al. (1983), Klich (2002), Samson and Frisvad (2004) and Samson et al. (2004)*.

Mycotoxicological investigation. AB1, OTA and ZEA in feed, as well as AM1 in raw milk samples, were determined by VICAM fluorimetric method using procedures for their identification *(Afla B^{TM}, Ochra Test^{TM}, Zearala Test ^{TM}, Afla M1^{TM})*.

Results and Discussion

Mycological investigations. About 91% of all tested samples were contaminated with moulds during the year. The presence of moulds was observed

in all samples of concentrate, fresh alfalfa, dried alfalfa, hay, pelleted and fresh sugar beet pulp, fresh beet leaf, fresh beet and pelleted malt spent grains. Lower contamination was observed the samples of fresh corn stover (50%) and corn silage (summer and winter – 67%, autumn – 83%).

Total viable mould count ranged from 10 (corn silage – spring) to 4.9 x 10^6 per gram (dried alfalfa – winter) – Table 1.

Table 1. Total viable mould count per 1 g of cattle feed samples

Feed	Summer	Autumn	Winter	Spring
Concentrate	$3.0x10^3$-$3.8x10^5$	$1.8x10^4$-$6.1x10^4$	$1.4x10^4$-$9.5x10^4$	$2.1x10^4$-$2.9x10^5$
Fresh alfalafa	$2.6x10^5$	$1.3x10^5$	-[a]	-
Dried alfalfa	$2.6x10^3$-$1.1x10^5$	$2.7x10^4$-$2.1x10^6$	$1.7x10^4$-$4.9x10^6$	$8.5x10^3$-$2.2x10^5$
Hay	$2.0x10^3$-$4.8x10^4$	$4.1x10^4$-$7.3x10^5$	$5.2x10^3$-$1.7x10^5$	$2.6x10^3$-$4.1x10^5$
Corn silage	$1.1x10^2$-$1.6x10^2$	$1.5x10^2$-$1.0x10^3$	25.0-$3.1x10^4$	10.0-$4.0x10^4$
Fresh corn stover	$2.0x10^3$	-	-	-
Pelleted sugar beet pulp	$2.0x10^2$-$3.1x10^3$	-	$9.0x10^2$-$2.1x10^4$	$4.0x10^2$-$1.4x10^4$
Fresh sugar beet pulp	-	$2.5x10^5$	-	-
Fresh sugar beet leaf	-	$1.4x10^5$	-	-
Fresh beet	-	-	$1.8x10^2$	-
Pelleted malt spent grains	$1.9x10^2$-$5.0x10^3$	30.0	$4.5x10^2$-$2.0x10^3$	$1.7x10^3$
Sunflower meal	$3.8x10^5$	-	-	-

[a] feed was not used

The moulds isolated from feed samples belong to 20 genera and 72 species (Table 2). The highest number of different species was isolated from concentrate (44), dried alfalfa (39) and hay (27), while the lowest was found in fresh corn stover (3) and fresh sugar beet pulp (2). However, it should be taken into account that only 2 samples of fresh sugar beet pulp (in autumn and winter) and 2 samples of fresh corn stover (in summer) were used for dairy cattle feed during the research year, while the number of concentrate, dried alfalfa and hay samples was considerably higher.

The highest frequency in feed mycopopulations showed species *Penicillium aurantiogriseum*, *Mucor hiemalis* and *Alternaria alternata*, that have been isolated from 45%, 32% and 23% of all tested samples, respectively.

Table 2. Fungal species isolated from dairy cattle feed samples in all seasons

Species	Summer	Autumn	Winter	Spring
Absidia ramosa	+	+	+	+
Alternaria alternata	+	+	+	+
A.chlamydospora	+	-	-	-
Aspergillus candidus	+	-	-	+
A.caespitosus	-	+	-	-
A.clavatus	+	+	-	-
A.flavus	+	+	+	+
A.fumigatus	+	+	+	+
A.nidulans	-	+	-	-
A.niger	+	+	+	+
A.ochraceus	-	+	-	-
A.oryzae	+	-	-	-
A.sclerotiorum	+	-	-	-
A.sydowii	+	-	-	+
A.terreus	-	-	+	-
A.ustus	-	+	-	-
A.versicolor	-	+	+	+
A.wentii	+	+	-	-
Cladosporium cladosporioides	+	+	+	+
C.herbarum	+	+	-	+
C.oxysporum	+	-	-	-
C.sphaerospermum	-	+	-	-
Cunninghamella sp.	-	-	-	+
Eurotium herbariorum	+	+	+	+
Fusarium equiseti	+	-	-	+
F.flocciferum	+	-	-	-
F.fusarioides	-	+	-	-
F.moniliforme	-	+	-	-
F.oxysporum	+	+	+	-
F.semitectum	-	+	+	-
F.solani	+	-	-	-
F.tabacinum	+	+	-	-
F.tricinctum	-	+	-	-
Geotrichum candidum	-	-	+	+
Gilmaniella humicola	+	-	-	-
Mucor christianiesis	-	+	+	-
M.circinelloides	+	+	-	+
M. dimorphosporus	-	-	+	-
M.hiemalis	+	+	+	+
M.petrinsularis	+	-	-	-
M.racemosus	-	-	+	-
M.rouxianus	+	-	-	-
M.sinensis	+	-	-	+
Oidiodendron tenuissimum	+	-	-	-
Phoma pomorum	+	+	+	-
Penicillium aurantiogriseum	+	+	+	+

P.capsulatum	-	-	-	+
P.claviforme	+	-	+	+
P.chrysogenum	+	+	-	+
P.commune	+	+	-	-
P.decumbes	+	-	-	-
P.echinulatum	-	-	+	-
P.expansum	-	-	-	+
P.fellutantum	+	-	-	-
P.frequentans	-	-	-	+
P.funiculosum	-	+	-	+
P.hordei	-	-	+	-
P.islandicum	-	+	-	+
P.rugulosum	-	+	-	-
P.sublateritum	-	-	+	-
P.variabile	+	+	-	-
P.verrucosum	-	-	-	+
Rhizopus cohnii	-	-	+	-
R.stolonifer	+	+	+	+
Scopulariopsis brevicaulis	+	+	+	+
Stagmanospora samarorum	+	-	-	-
Stemphilium solani	+	-	-	-
S.vesicarium	-	+	-	-
Trichoderma harzianum	+	-	-	-
T.koningii	+	-	-	-
T.viride	-	+	+	-
Verticillium albo-atrum	-	+	-	-

The most of isolated species, particularly from genera *Aspergillus*, *Fusarium* and *Penicillium*, are reported to be potential producers of different toxic metabolites (*Samson et al., 2004; Krnjaja et al., 2011a,b)*. Besides, some of the isolated species (*Aspergillus fumigatus, Cladosporium herbarum, Alternaria alternata,* etc.) produce metabolites that are reported to be causative agents of different allergic reactions in humans *(Horner et al., 1995, Škrinjar et al., 2009)*.

Mycotoxicological investigations showed that 42 samples of total 98 tested, were contaminated with mycotoxins (Table 3). The most frequent mycotoxin in feed samples was OTA, whose residues were found in 24 samples, in concentrations from 20.00 (corn silage – spring) to 210.00 μg/kg (fresh alfalfa – summer). ZEA was detected in 12 samples (250.00 – 980.00 μg/kg) and AB1 in 3 samples (traces – 18.50 μg/kg).

None of the tested raw milk samples was contaminated with AM1.

Table 3. Presence of AB1, OTA and ZEA in diary cattle feed samples

Feed	AB1 (μg.kg^{-1})				OTA (μg.kg^{-1})				ZEA (μg.kg^{-1})			
	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp
Concentrate	-[a]	-	-	-	43.30	48.45 82.20	82.00 20.50	20.30	-	-	-	250.00
Fresh alfalfa	-	-	-	-	42.00 210.00	-	-	-	-	-	-	-
Dried alfalfa	-	-	-	-	45.50 83.30 82.00	-	-	-	-	-	-	-
Hay	-	-	-	-	44.10 65.00 56.20	-	-	-	-	-	-	-
Corn silage	-	-	-	-	45.00 45.50	85.50 86.20	21.20	20.00 21.50 45.40	-	-	500.00	500.00
Fresh corn stover	-	-	-	-	-	-	-	-	250.00	-	-	-
Pelleted sugar beet pulp	-	-	-	18.50	81.15 44.00	-	84.50 82.50	17.50	250.00 245.00	-	980.00 980.00	510.00 500.00 450.00 250.00
Fresh sugar beat pulp	-	-	-	-	-	-	-	-	-	-	-	-
Fresh sugar beet leaf	-	-	-	-	-	-	-	-	-	-	-	-
Fresh beet	-	-	-	-	-	-	-	-	-	-	-	-
Pelleted malt spent grains	-	traces	14.00	18.50	-	-	-	-	-	-	-	250.00
Sunflower cake	-	-	-	-	-	-	-	-	-	-	-	-

S – summer, A – autumn, W – winter, Sp – spring;
[a] toxin was not detected

Conclusion

This study has identified mould species that contaminated different dairy cattle feed samples throughout one research year (in all seasons). Diversity of isolated mycopopulations was very high (20 genera and 72 species). The most of isolated moulds are reporterd to be capable of some toxins and alergens biosynthesis. The contamination of feed samples with mycotoxins was observed in 42.8% of all tested samples. All raw milk samples tested were aflatoxin M1 free. Even that detected concentrations of mycotoxins were mostly in acceptable range for cattle feed, the presence of mycotoxins and mycotoxin-producing moulds in feed poses a high risk for animal and human health.

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Rezultati jednogodišnjeg istraživanja kontaminacije hrane za krave muzare i sirovog mleka plesnima i mikotoksinima

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Rezime

U radu je ispitana kontaminacija 98 uzoraka hrane za ishranu krava muzara plesnima i mikotoksinima (AB1, OTA, ZEA). Pored toga je ispitana i kontaminacija 80 uzoraka sirovog mleka aflatoksinom M1 (AM1). Određivanje ukupnog broja plesni po gramu hrane izvršeno je pomoću standardne metode po Kohu, dok je identifikacija fungalnih vrsta urađena prema ključevima koje su dali autori: *Elis (1971, 1976)*, *Pidopličko i Miljko (1971)*, *Nelson i sar. (1983)*, *Klih (2002)*, *Samson i Frisvad (2004)* i *Samson i sar. (2004)*. Sadržaj AB1, OTA i ZEA u uzorcima hrane, kao i AM1 u sirovom mleku, određeni su VICAM fluorometrijskim metodom. Rezultati su pokazali da je 91% ispitivanih uzoraka bilo kontaminirano plesnima, a ukupan broj plesni je bio od 10 (kukuruzna silaža – proleće) do 4.9 x 10^6/g (suva lucerka – zima). Izolovane plesni svrstane su u 20 rodova i 72 vrste. Rezultati mikotoksikoloških ispitivanja su pokazali da je 42 uzorka od 98 ispitanih bilo kontaminirano mikotoksinima. Najfrekventniji je bio OTA (24 uzorka, 20.00 - 210.00 μg/kg), sledi ga ZEA (12 uzoraka, 250.00 – 980.00 μg/kg) i na kraju AB1 (3 uzorka, tragovi – 18.50 μg/kg). Aflatoksin M1 nije pronađen ni u jednom uzorku sirovog mleka.

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