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MYCOTOXINS IN THE SILAGE — CAUSES OF CREATING, AFTERMATH AND PROTECTION FROM ACTING

ABSTRACT: The causes of appearance of mold in the silage, genus and species of fungi which biosynthesize mycotoxins, acting consequences of micotoxins, prevention of fungi growth and possibilities to prevent their negative effects are shown in this paper. Also, the results of mycotoxins presence in the silage (corn and lucerne) in Vojvodina in the period 2000—2004 are presented. The most commonly found mycotoxins were zearalenone in 60.6% of analyzed samples and DAS in 30.3% of samples. Silage contamination with ochratoxin A, aflatoxin B1 and T-2 toxin was between 15.2 and 21.2%. The content of mycotoxins DAS and T2 toxin was above the values allowed by regulative. The solutions which contribute to the prevention of development of the molds and elimination of negative effects of mycotoxins in silage were analyzed and suggested.

KEY WORDS: adsorbents, helath, mycotoxins, silage

INTRODUCTION

Silaging of animal feed is a possibility for keeping the quality of feed in fresh stage for a long time. That is happening because of the presence and activity of lactic acid, acetic acid and butyric acid bacteria. Besides them, in the ensilig material the molds and yeasts which produce numerous metabolites are present. The main bacteria activity product in the silage is lactic acid which is at the same time most favourable one, too. In the silage material, some minor quantities of acetic acid, butyric acid and propionic acid, ethyl alcohol, carbon dioxide and ammonium appear. Acetic acid, butiric and propionic acid are weak conservators and, at the same time, indicators of undesirable processes in the silage, like missing in ensiling. These acids, especially propionic acid, act as fungicides, in order to prevent molds growth, compared to a lactic acid, so in some cases they are favourable in the silage. Because of that, propionic acid is used during the storage of animal feed.

Silage is very favourable environment for molds development. Humidity in the silage, depending on the type of plant material, is from 30 to 80%, temperature from 5 to 40°C, while concentration and availability of the nutritive materials is high. Molds may already biosynthetize mycotoxins in the growth phase of plants which during the silage preparation are carried into the silo. Molds are mostly active in the environment with oxygen, so they grow on the surface of silage or in the parts where the air is not pressed out enough. Compared with lactic acid bacteria, molds endure much higher acidity and may be active in the environments which have pH between 2 and 3 or lower. The most common genus/species of molds and their mycotoxins in silage (Chadd, 2004) are shown in Table 1.

Table 1. Common molds in European silage and their mycotoxins (Chadd, 2004)

Silage	Genus	Species	Toxin
Grass Silage	*Penicillium*	*roqueforti*	Roquefortin
	Aspergillus	*fumigatus*	Aflatoxin
	Aspergillus	*flavus*	Aflatoxin
	Fusarium	*moniliforme*	Fusaric acid
Maize silage	*Fusarium*	*graminearum*	Zearalenone
	Fusarium	*roseum*	Fumonisins B_1, B_2
	Fusarium	*verticillioides*	Fumonisins B_1, B_2
	Fusarium	*moniliforme*	Ochratoxin A
	Penicillium	*verrucosum*	Ochratoxin A

DISORDERS CAUSED BY MOLDS AND MYCOTOXINS

Harmful effects of molds on animal health may be seen in a form of illness-mycosis, while the influence of their mycotoxins manifests in a form of intoxications — mycotoxicoses. The most frequent mycosis is aspergillosis caused by mold from genius *Aspergillus* (*A. fumigatus, A. flavus, A. nidulans, A. niger, A. terreus*), as well as by molds form genuses *Mucor* i *Absidia* which are commonly found in hay.

According to their activity, mycotoxins may be: hepatotoxins, nephrotoxins, neurotoxins, citotoxins, estrogen toxins, immunosupresive toxins and photosensibile toxins (Sinovec et al., 2003). In this work more attention was payed to the influence of mycotoxins on cuds, because silage in their feed is the biggest portion of diet.

In beef and sheep mycotoxins cause lower consumption and feed uptake, decreasing of milk yield, weaker growth of socket in fat and rejuvenate, high number of abortuses, not regular and not enough expressed estrus, decreasing the rate of pregnancy, retaining of the endometrium, uterus inflammation, dislocation of abomasus, ketosis, appearance of the oily degeneration of liver, weaker immunity, higher number of somatic cells in milk and accumulation of residua of mycotoxins in milk and meat. The cattle is less sensitive because microorganisms of rumen with their enzymes degradate the mycotoxins and thus decrease their negative effects (Whitlow et al., 2004) The most common molds are from genus *Penicillium, Aspergillus* and *Fusarium* (Fink-Gremmels, 2004; Auerbach, 2003; Mašić et al., 2003 b).

Penicillium. From the kinds of this genus the most frequent is *P. roquerforti.* It biosynthetizes toxins rokvefortin C, patulin, mycofenol acid, PR toxin and penicillin acid. Ensiling material may be naturally contaminated by some of these mycotoxins (Fink-Gremmels, 2004). Penicillin acid is the first metabolite which appears after ensiling (13 days), followed by patulin (22—27 days), mycofenol acid (36 days) and PR toxin (49 days).

Rokvefortin C and patulin are neurotoxins which in the cattle cause muscle weakness and disarrangement of coordination. Symptoms are not specific in the cattle because they are more resistant to these mycotoxins. Cattle may be more sensitive to mycofenol acid, penicillin acid and PR toxin. They may have negative influence on development of the microorganisms population in the rumen and cause local inflammatory processes. Toxins which are biosynthetized by *P. rubrum* may cause hemorrhagic disfunctions in blood capillaries in the cattle. *P. citrinum* biosynthetizes citrine and the symptoms of this toxin poisoning are laxity renal insufficiency and uremia which in some cases finish by death. The autopsy usually show changes in the mouth, oesophagus and omasum, especially in the form of perirenal edem. Molds from genus *Penicillium* produce the ochratoxin A which does not cause severe consequences in grown cattle, because in the large amount (more the 50%) this toxin is degraded by microorganisms of rumen.

Aspergillus. Molds of this genus are very often found in the silage. The most common is *A. fumigatus* which biosynthetizes micotoxins: verukologen, fumitremorgen and peniterm. These toxins cause tremor and other kinds of neurotoxicology in sheep. Symptoms of intoxication are difficult to notice in the farm conditions, in contrast to intoxications with lolitrems and ergovalins which causes *Neotyphodium* mold. Disposal of animals to the spores of *A. fumigatus* cause bronchitis, mastitis and abortus of cattle. *A. flavus, A. parasiticus* and *A. nomius* biosynthetize extremely toxic mycotoxins (aflatoksin B_1, B_2, G_1, G_2) which cause the symptoms characteristic for aflatoxicoses — weak growing up, leaning and decreasing the milk yield and lead to the harder kind of indigestion disturbed work of browels, neuro disarrangements, damaging the capillaries and dysentery. If the poisoning is heavy, the death comes in 1—3 days. Compared to cattle, sheep are more resistant to aflatoxins. Between 0.3 and 6.2% of dietary aflatoxins B1 and B2 were found in the milk of cows in a form of aflatoxins M1 and M2 exuding within 4—5 days after consumption of contaminated feed. Also, some special types of molds from the genus *Aspergillus*, biosynthetize the ochratoxin A, which, thanks to the activity of microorganisms in rumen, does not cause severe damages.

Fusarium. Molds of this genus biosynthetize mycotoxins zearalenone and trichothecenes from which the most common are: deoxynivalenol or vomitoxin (DON), diacetoxiscirpenol (DAS), T_2-toxin and fumonisins B_1 i B_2. Zearalenone is biosynthetized by species *F. graminearum, F. culmorum, F. oxysporum* and others. This toxin may bind estrogen receptors, cause hyper estrogenism and lower fertility. Trichotecenes mycotoxins are produced by *F. graminea-*

rum, F. culmorum, F. tricinctum, F. sporotrichioides, F. poae, F. semitectum, F. nivale and other species of the same genus. Also, trichotecenes are biosynthetized by other genus of fungi: *Trichothecium, Trichoderma, Cephalosporium* etc. These toxins have influence on weak feed consumption, damage the mucous membranes of rumen and rennet, appearence of laxity, hemorrhagic changes on the heart, kidneys, spleen, and urinal bubble, and act as the immune suppressives. Till now, not enough investigated toxins fumonizins B_1 and B_2 are produced by species *F. verticillioides and F. proliferatum;* these toxins may damage the liver and decrease the milk production in cows.

PRESENCE OF MYCOTOXINS IN SILAGES IN OUR COUNTRY

The most common producers of mycotoxins in Serbia are molds of genus *Fusarium*, particularly species *F. graminearum, F. verticillioides, F. subglutinans, F. oxysporum* i *F. proliferatum*. They are isolated on the corn and grain. Besides these species, on lucerne there are isolated other species of molds from the same genus, like *F. avenaceum, F. tricinctum, F. sporotrichioides, F. solani* (Krnjaja et al., 2004). The most common toxin which they biosynthesize is zearalenone. Besides zearalenone, the most often isolated is T-2 toxin, as well as the other trichotecenes. In the first place it is DAS which follows T-2 toxin and DON which follows zearalenone. Mašic et al. (2003a), in investigated corn samples in Serbia, fortified the presence of zearalenone in 36,88% of samples (average concentration 750 µg/kg), trichotecenes in 31,82% of samples (240 µg/kg), ochratoxin A in 27,05% of samples (69 µg/kg) and aflatoxin B_1 in 19,67% of samples (36 µg/kg). The quantity of mycotoxines in the silage originated from part of Vojvodina, fortified by Bočarov-Stančić, 2005 are shown in Tables 2 and 3.

An official tolerance level of mycotoxins in feed and mixture are allowed by Regulations on maximal permitted quantities of harmful materials and components in cattle feed (Official Gazette SRJ, br.2/90). According to these regulations, however, no tolerance level of mycotoxins in silage is given.

Table 2. Mycotoxins in silage samples in Vojvodina region, for the period 2000—2004 (Bočarov-Stančić, 2005)

Silage	*Aflatoxin*		*Ochratoxin A*		*Zearalenone*	
	Contamination sample, %	Average, µg/kg	Contamination sample, %	Average, µg/kg	Contamination sample, %	Average, µg/kg
Corn plant	25	3	37,5	75	43,8	1450
Corn dent	0	0	9,1	130	63,6	1640
Lucerne	16,7	3	0	0	100,0	730

Table 3. Mycotoxins (trihotecens) in silage samples in Vojvodina region for the period 2000—2004 (Bočarov-Stančić, 2005)

Silage	*T-2 toksin*		*DAS*	
	Contamination sample, %	Average, μg/kg	Contamination sample, %	Average, μg/kg
Corn plant	25	310	43,8	620
Corn dent	27,3	500	18,2	1380
Lucerne	0	0	16,7	250

POSSIBILITIES OF PROTECTION FROM MOLD AND MYCOTOXINS ACTION

Protection from mold and mycotoxins may be done in three basic phases: 1) on the arable — regular crop rotation, optimal terms of works, plant protection by fungicides and insecticides, using healthy seed, making more resistant genotypes of plants on development the molds, destroying the damagers etc. 2) during the ensiling — conveying adequate technology of silaging, using bacterial-enzyme inoculants, agents against developing of the mold, protection against progressing the air, water, rodents and other kinds of protection 3) during the usage of silage — nutritive intervention in the diet like increasing the content of energy and proteins additive to probiotics, enzymes, vitamins, amino acids and anti oxidants, adsorbents of mycotoxins from organic or mineral origins. Very efficient and rational way to prevent the negative effects of mycotoxins is focused on the application of inorganic mineral adsorbents based on natural zeolites of domestic origin. They can be used in the phase of ensiling, apropos using the silage if there is fortified presence of mycotoxins in it. The new mineral adsorbent of mycotoxins obtained by cation exchange of inorganic cations on the zeolite surface with organic cations ("Min-a-Zel Plus") is produced in the Institute for the Technology of Nuclear and Other Mineral Raw Materials, Belgrade. The addition of "Min-a-Zel Plus" to the green mass of plant of corn during the ensiling (0.2% from the mass of plant) has influence on increased of production of lactic acid, decreasment of the number of molds and mycotoxins in the silage (zearalenon, T-2 toxin and DAS), as well as the ammonia nitrogen (Table 4).

Table 4. Efficiency of "Min-a-Zel Plus" in silaging of corn (Adamović et al., 2001)

Parametar	Without "Min-a-Zel Plus"	With "Min-a-Zel Plus"
Dry mater, %	22,34	24,28
Lactic acid, %	49,52	75,40
Acetic acidd, %	50,48	24,61
pH	3,36	3,40
NH_3-N	0,03	0,02
Total mold in 1 g	1000	100
Zearalenone, mg/kg	1,02	0,90
T-2 toxin, mg/kg	0,50	0,25
DAS, mg/kg	0,50	0,00

Đorđević et al. (2003) and Đorđević et al. (2004) reported the similar results for application of products based on zeolite during the ensiling of sugar beet pulp. Additional regulations and procedures of protection from mycotoxins in the silage may be: regular organoleptic and laboratory valuation of the silage, bringing law regulations about limited quantities of mycotoxins in the silage, harmonizing the methods and the procedures for determination of mycotoxins, equiping the laboratories, education of laboratory personnel and development of the efficient procedures and instruments for protecting the animals from mycotoxins.

CONCLUSION

Protecting against molds and mycotoxines in the silage needs to start just on arable and to continue in the process of silage, apropos using the silage. Regular control supported by law regulations may have important contribution to the success in the fight against mycotoxins.

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МИКОТОКСИНИ У СИЛАЖИ — УЗРОЦИ СТВАРАЊА, ПОСЛЕДИЦЕ И ЗАШТИТА ОД ДЕЛОВАЊА

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Резиме

У раду се указује на узроке појаве плесни у силажи, родове/врсте плесни које биосинтетишу микотоксине, последице њиховог деловања, мере спречавања развоја и могућности отклањања штетног утицаја. Истакнуто је да услед пропуста у технологији силирања (недовољно гажење, присуство ваздуха) при влажности масе за силирање, постоје повољни услови за развој плесни које биосинтетишу токсине штетне по здравље животиња и људи. Они могу да изазову здравствене поремећаје укључујући и патохистолошке промене на органима и ткивима, депонују се у производима (млеку, месу и јајима) и на тај начин угрозе здравље људи. У раду су изложени резултати присуства микотоксина у силажи (кукуруз и луцерка) у Војводини у периоду 2000—2004. Најчешће присутан микотоксин био је зеараленон у 60,6% анализираних узорака, а потом ДАС у 30,3% узорака. Контаминираност силаже охратоксином А, афлатоксином Б1 и Т-2 токсином била је између 15,2 и 21,2%. Садржај микотоксина ДАС и Т-2 токсина био је на граници или изнад вредности које дозвољава *Правилник* (Службени лист СРЈ, бр. 2/90). Вредности за остале микотоксине биле су испод максимално дозвољених количина. Анализирана су и предложена решења која доприносе сузбијању развоја плесни и микотоксина.