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Mycotoxins in Silages: Occurrence and Prevention

Review Article

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ABSTRACT

Mycotoxins are an increasingly discussed topic. Several scientific reports have been written which review the effects of these toxic substances on the health and productivity of animals. However, there is a lack of work regarding the incidence of mycotoxins in ensiled material and the consequences of this occurrence. In this review, practical and field information was converged with scientific data with the objective of clarifying the subject. Nevertheless, due to the complexity of this topic, a straight line was adopted, starting with a brief explanation about mycotoxins followed by some practical prevention methods used on the field and during the ensiling process and, very importantly some analyses' results made to silage from various origins and the impact of these contaminated materials in the animals ingesting them. An overview about how to avoid the negative impact of these poisonous substances is given as, for example, on the field (crop rotation, use of resistant plants, use of fertilizers, biological and chemical control of fungi and adequate plant maturation); in the silo (compaction, sheeting, storage length) and a correct management of the open silo (speed of progression in the silo, discard of deficiently preserved silage, additional use of silage additives for stopping the undesirable fermentations).

KEY WORDS impact, mycotoxins, occurrence, prevention, silage.

INTRODUCTION

In spite of the use of many preventive methods on the field, during harvest and storage, complete mycotoxin elimination is far from reality. Even the best management of agricultural strategies cannot totally eradicate mycotoxin contamination (Jouany, 2007). In fact, with the extreme, mycotoxin-stimulating weather conditions (namely heavy rains and/or drought) occurring more frequently worldwide, mycotoxin contamination is becoming an increasingly disturbing phenomenon.

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a negative response on animals and humans, either by ingestion, inhalation or skin-contact. Although more than 70,000 species of fungi have been described, only some of them are able to produce mycotoxins. *Fusarium*, *Aspergillus* and *Penicillium* are the genus which are producing the most hazardous mycotoxins in terms of agricultural and animal production: trichothecenes (namely T-2 toxin (T-2) and deoxynivalenol (DON)), zea-ralenone (ZON), fumonisins (FUM), aflatoxin B_1 (AfB1) and ochratoxins (OTA) (Table 1).

According to Richter and Bauer (2007), the most frequently occurring mould in corn silage is *Penicillium roqueforti*, whereas, in grass silages *Monascus ruber* and *Aspergillus fumigatus* are the most common. The last two moulds were classified by Pelhate (1977) as tolerant in their tolerance to oxygen, whereas, *Penicillium roqueforti* is considered as microphilic or indifferent to oxygen presence. Also Pelhate (1977), Veselý *et al*. (1981), Amend (1990), Addler (1993), Skaar (1996), Auerbach *et al*. (1998) found in different regions of Europe (France and Italy, former Czecho-

Table 1 Classification of mycotoxin-producing fungi (Weidenbörner, 2001; Richard and Payne, 2003)

Major classes of mycotoxin-producing fungi	Fungi species	Mycotoxins
Aspergillus	*A. flavus* *A. parasiticus* *A. nomius* *A. pseudotamarii*	Aflatoxin (B_1, B_2, G_1, G_2)
	A. ochraceus	Ochratoxin (Ochratoxin A)
	A. clavatus *A. terreus*	Patulin
	A. flavus *A. versicolor*	Cyclopiazonic acid (CPA)
Claviceps	*C. purpurea* *C. fusiformis* *C. paspali* *C. africana*	Ergot alkaloids: Clavines (Argroclavine) Lysergic acids Lysergic acid amids (Ergin) Ergopeptines (Ergotamine, Ergovaline)
Fusarium	*F. verticillioides* *(syn. F. moniliforme)* *F. proliferatum*	Fumonisin (B_1, B_2, B_3)
	F. graminearum *F. avenaceum* *F. culmorum* *F. poae* *F. equiseti* *F. crookwellense* *F. acuminatum* *F. sambucinum* *F. sporotrichioides*	Type A Trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol Type B Trichothecenes Nivalenol, deoxynivalenol, fusarenon-X
	F. graminearum *F. culmorum* *F. sporotrichioides*	Zearalenone
Penicillium	*P. verrucosum* *P. viridicatum*	Ochratoxin (Ochratoxin A)
	P. citrinum *P. verrucosum*	Citrinin
	P. roqueforti	Roquefortine
	P. cyclopium *P. camemberti*	Cyclopiazonic acid (CPA)
	P. expansum *P. claviforme* *P. roquefortii*	Patulin
Neotyphodium *(formerly Acremonium)*	*N. coenophialum*	Tall fescue toxins: Ergot alkaloids, lolines, peramine
	N. lolii	Tall fescue toxins: Lolitrems, peramine, ergot alkaloid (ergovaline)

slovaquia, Germany, Austria, Germany, respectively) the predominance of the contamination with *Penicillium roqueforti* in grass and corn silages.

Minimizing mycotoxin contamination in the field

Since more than 90% of the mycotoxins in the feed are already produced on the field, the first step to avoid mycotoxins in the silages should be done at the site of crop production. Richter (2008) found from 1×10^4 up to 1×10^6 cfu per gram of black fungi in the ephyfitic microflora of forage crops. Several environmental factors play a role in the growth of the moulds in the field: temperature, composition of the gas atmosphere, substrate properties including moisture content and water activity (a_w), pH and chemical com-

position, as well as biotic factors (insects, vertebrates and other microorganisms) (Ramakrishna *et al*. 1993; Ominski *et al*. 1994).The use of resistant plants against *Fusarium spp*. is recommended, as well as a good crop rotation. Rain and high thermal amplitude are supporting risk factors, therefore, the weather forecast or the weather conditions should be known as they are valuable sources of information concerning the risk management. Authors like Oldenburg (2006) recommend, not using direct drilling for cereals when the preceding crop was corn in order to prevent *Fusarium* contaminations, a suggestion supported by the results of Dill-Macky and Jones (2000), because of the similarities between those crops. Obst *et al*. (1997) reported that wheat following corn promotes a higher contamination with DON. Edwards (2004) widely reviewed the influence of different factors such as the choice of fertilizers and chemical and biological control of insects, weeds and fungi, in the contamination of grain by trichothecene mycotoxins.

The level of field mycotoxins is known to increase with plant maturation. Therefore, authors like Jones *et al*. (1981); Warfield and Gilchrist (1999) support the need for an adequate planning of harvesting activities.

Avoiding mycotoxin contamination in the ensiling process

While, fusariotoxins are mostly produced on the field, *Aspergillus* and *Penicillium* fungi will most likely develop after harvest leading to the production of aflatoxins and ochratoxins, especially in poor storage conditions. However, as most of the toxic compounds present on the agricultural commodities will remain stable after harvest under aerobic conditions (Scudamore and Livesey, 1998), crop management should not be discarded as an important factor.

As referred earlier, the next important step after harvest is the storage procedure. A deficient storage can lead to the deterioration of feed quality. Whenever, feedstuffs are stored for a certain period of time (weeks or months), moulds can grow on their surface and inside. The consequences of this occurrence are high losses of dry matter and nutrients, fungal growth and mycotoxin production (Kalac and Woodford, 1982), all of which have negative impacts on animal performance and health. Ensiling has become an important process for the conservation of harvested crops. Its aim is to preserve the feedstuffs' nutrients, making them available throughout the year. This process is based on the anaerobic storage in order to promote the growth of desirable microorganism (lactic acid bacteria that lead to a deep acidification) and to prevent the contamination with undesirable microorganisms (especially *Clostridium spp.* and *Listeria spp.* bacteria, moulds and yeasts; Kalac and Woodford, 1982).

The management of the silage is crucial to diminish the risk of contamination caused by moulds. Therefore, good practices during silage preparation and feed out phase management are essential to avoid mould growth and mycotoxin formation in the ensiled material.

The hygiene (clean crop and clean silo) should be maximized, since dirt can considerably increase the number of undesirable microorganisms, namely *Clostridia* and *Listeria*, and fungi due to the ubiquitous existence of *Fusarium* spores in the soil (Schrödter, 2004). Producers can overcome this situation, for instance, by controlling the cutting height of the harvester.

According to Scudamore and Livesey (1998), the field-derived fungi will, in time, be replaced by storage fungi, particularly with inadequate drying or if the moisture content is not maintained below 15%. In the case of silages, the moisture content is 3 to 5 times higher than this value and therefore water activity (a_w) is much higher than needed by fungi (0.65 according to the same authors), which will increase the contamination risk. The most common silage materials are grass, corn, whole crop cereals and different industrial by-products. Authors like Richter *et al*. (2005) gave provisional orientation values for the contaminations with moulds in corn and grass silages (Table 2).

All these are known sources of moulds (mainly *Fusarium* in the field) and mycotoxins (trichothecenes, zearalenone and fumonisins), which will then persist in the silages. In spite of the fact that some moulds can grow even under anaerobic conditions/low amount of oxygen, the creation of anaerobic conditions in the silage can reduce consider-ably the growth of fungi and therefore mycotoxin formation. Two aspects are essential to reach and to control the oxygen entrance into the silage: compaction and coverage. Compaction eliminates the oxygen inside the material and coverage maintains the silage anaerobically preserved.

When the silage is well compacted, the oxygen entrance and penetration will be limited to the layer in contact with the air in the feed out phase (Losand, 2003) (Figure 1) and

Table 2 Provisional orientation values for mould contamination in corn and grass silages (adapted from Richter *et al*. 2005)

Mould and black fungi		Corn silage	Grass silage
Product typical mould and black fungi	*Acremonium* *Verticillium* *Fusarium*	5×10^3	1×10^4
Spoil indicating mould and black fungi	*Aspergillus* *Penicillium* *Scopulariopsis* *Wallemia*	1×10^4	1×10^4
Mucorales		3×10^3	5×10^3

the aerobic stability will be improved (Kleinmans, 1996).

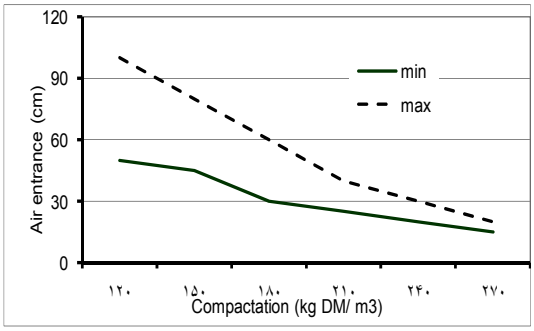

Figure 1 Relationship between air entrance on the surface of maize silages and compaction (adapted from Losand 2003)

Crop particle length is closely related with compaction. The "rule of thumb" at this stage is: the drier the material to be ensiled, the smaller the crop particle should be.

The coverage of the ensiled matter should be done immediately with plastic sheets (polyethylene). It is very important to use exclusive adequate sheets especially for this purpose. A low quality sheet will permit air penetration and enable mould growth and further production of mycotoxins, also leading to losses of dry matter and energy content. An extended silage practice in Middle Europe is to use bags filled with sand for fixing the sheets and support the silo hermeticity. Once the silo is air tight, respiration stops and fermentation can be initiated.

Although, not useful in preventive situations due to its highly questionable efficacy, storage length is considered by some authors to have an impact on the mycotoxin content. noted that aflatoxin B_1 is susceptible to breakdown under ensiling conditions, depending on pH, temperature and length of storage; nevertheless, the mode of action is not clear. Richter (2006) reported a decrease in ergot alkaloids produced by *Claviceps purpurea* during storage. Zearalenone and some of the trichothecenes appear not to be affected by anaerobic and acid conditions in silage (Lepom *et al*. 1988). Rotter *et al.* (1990) reported that although ochratoxin is apparently degraded when the contaminated grain is ensiled, its toxic effect remains. Given these contrasting results and taking into account the known and thoroughly studied toxicity of these molecules, the threat of mycotoxins should not be disregarded.

Ensiling time plays an important role also in case of acetic acid fermentation. Silage is a rich source of nutrients (starch, lactic acid and amongst others) for yeasts and moulds and can therefore become unstable if in contact with oxygen. The aerobic stability (time the silage remains stable in contact with air, also known as bunk or silage shelf life) can be enlarged using appropriate silage additives (heterofermentative bacteria-acetic or propionic acid producers-or organic acids directly applied on the surface contacting the air), which will stop the growth of yeasts and moulds, and subsequently avoid the production of mycotoxins. The production of acetic acid in the silage begins later than that of lactic acid. That is the reason why it is crucial to wait the appropriate time (at least 4 to 6 weeks) until the heterofermentative bacteria ferment sugars, and in many cases, part of the lactic acid, into acetic acid. Nußbaum (2005) considers a minimum of 6 to 8 weeks as an adequate time for a proper acetic acid (and propandiol) production. Usually during the ensiling process, temperature increases, meaning that aerobic spoilage is taking place. Silages are less stable if they contain residual sugar or starch. A temperature increase of 2 ºC (laboratory conditions) to 5 ºC (practical conditions) (Nußbaum, 2006) above room temperature can be considered a symptom of instability. Very often corn silages tend to be more unstable because of their high level of available nutrients. Muck and Bolden (1991) reported a faster growth of yeasts on corn silages.

To adequately manage the silo, the silage amount fed each week should guarantee an advance in the silo of 1.0 to 1.5 m and 2.0 to 3.0 m in winter and summer, respectively. This advance is related to the design of the silo and therefore its size must be carefully calculated. A suitable solution for an over dimensioned silo is to divide it in two halves by a wall (Figure 2). Another key factor to avoid mycotoxin contamination in the silo is to have a clean-cut face in the feed out phase, as this will give fewer opportunities for the growth of undesired moulds on the surface.

Figure 2 A silo divided by a wall in the middle to avoid aerobic spoilage under Brazilian practical conditions

The menace of mycotoxins in the silage

Mycotoxins have been isolated from silages that did not show visible mould contamination (Schneweis, 2000; Wilkinson, 2005; Acosta Aragón, 2010). Therefore, in practical terms, if by the one side it cannot be guaranteed that visually sound silage does not contain mycotoxins as these substances are odourless and invisible, if silage shows spoilage symptoms, it should not be fed to animals in order to pre-

vent the occurrence of mycotoxicoses, once there is a high probability of mycotoxin contamination in these cases. Instead, it is strongly recommended to discard the spoiled silage (30-40 cm of the surface). The fresh silage layer can be treated with acids (propionic acid, for instance) by spraying of 100 to 500 mL/m^2, in order to prevent further problems with the aerobic stability. Organic acids like acetic, propionic, butyric, benzoic and sorbic acid are potent mould inhibitors (Woolford, 1975; Lück, 1985; Clevström *et al*. 1989; Auerbach, 1996; Danner *et al*. 2003; Acosta Aragón *et al*. 2010). A negative correlation (r^2=0.61 for P<0.001) between undissociated acetic acid content and *Penicillium roqueforti* count at the end of anaerobic storage by Auerbach (1996). Some silage inoculants on the market are provided with heterofermentative lactic acid bacteria, which produce the antimycotic acetic acid and is responsible for a better aerobic stability of the silage (Driehuis *et al*. 2001).

Ideally and in order to make a good mycotoxin risk management, mycotoxin analysis should be performed to the feedstuffs and ultimately to the feed, prior to being fed to the animals. An important and difficult step in determination of mycotoxin contamination level in silages is the sampling procedure as these toxic substances do not occur homogeneously in the feedstuffs. Mycotoxin contamination within the silo is variable due to the heterogeneous cooling of the plants during storage and the formation of convection currents of warm air from the centre, that meet cool and damp atmospheric air at the tops and sides of the silo causing condensation, thus mould growth and mycotoxin production in specific areas named "hot spots" or nuggets (Figure 3, The numbers included in Figure 4 represent a hypothetical mycotoxin contamination, drawing the attention for the need of taking numerous samples in order to obtain more reliable results. Furthermore, other errors during this step bring about misleading results. For example, silage sampling tends to be selective, avoiding the worst material, and if this is made in newly opened silos, recontamination due the air exposure during the feed out phase is not being taken into account.

Figure 3 Representative scheme of the heterogeneity of mycotoxin contamination within a silo

Mycotoxin contamination in silages: analyses and potential risks

In spite of the producers' efforts to avoid or diminish the mycotoxin contamination of their silages, the problem is far from being solved. The toxic effects of mycotoxins vary widely, ranging from carcinogenicity, genotoxicity, dermonecroticity, nephrotoxicity, hepatotoxicity, oestrogenicity reproductive disorders and immunosuppression, depending on animal, environmental and mycotoxin factors (Dieckman and Green, 1992; Hoehler and Marquardt, 1996; Oswald and Comera, 1998; Riley *et al*. 1998; Shier, 1998; Bauer, 2002). In dairy cows, symptoms may include: reduced milk yield, reduced feed consumption, intermittent diarrhea, unthriftiness, rough hair coat, reduced reproductive performance including irregular estrus cycles, embryonic mortality, pregnant cows with estrus signs and decreased conception rates. Moreover, mycotoxin contamination generally leads to an increase of diseases such as displaced abomasus, ketosis, retained placenta, metritis, mastitis and fatty livers (Hussein and Brasel, 2001; Fink-Gremmels, 2008; Coppock and Jacobsen, 2009; Obremski *et al*. 2009). Due to their impact on the immune system, animals do not respond positively to veterinary therapy (Whitlow and Hagler, 2005).

As can be seen in Table 3, laboratory analyses of silages confirm the presence of mycotoxins in silage samples. Analyses were performed using standard procedures.

Table 3 Results of silage analyses in 2007 (Acosta Aragón and Rodrigues)

Parameter	Mycotoxin				
	AfB_1	ZON	DON	FUM	T-2toxin
No. tested samples	191	191	191	43	185
% positive	1.1	19.4	49.7	11.6	0.0
Maximum contamination level (µg/kg)	29	26728	1256	989	-
Average positive samples (µg/kg)	26.7	1211.8	241.7	498.0	-

Aflatoxins, ZON, DON and total FUM were analyzed by High Pressure Liquid Chromatography (HPLC), whereas, T-2 toxin values were obtained by Thin Layer Chromatography (TLC). For the purpose of data analysis, non-detect levels were based on the quantification limits of the test method for each toxin: Aflatoxin B1<0.5 µg/kg; ZON<10 µg/kg; DON<150 µg/kg; T-2 toxin<125 µg/kg and

FUM<25 µg/kg. More than 90% of the samples were sent from Japan and Australia.

The occurrence of AfB_1 and FUM was less frequent than that of others such as ZON and DON. None of the 185 samples were positive for T-2. Only 2 samples out of 191 (1.1%) were found positive for AfB_1. Scudamore and Livesey (1998) consider that surveillance for aflatoxin in silage and forages has rarely been reported, despite the acknowledged hazardous effects this mycotoxin implies. Aflatoxin degradation in the rumen is generally weak, inferior to 10% with dosages from 1 to 10 µg/mL (Yiannikouris and Jouany, 2002). Auerbach *et al*. (1998) also observed the formation of aflatoxicol, a hydroxylated highly toxic derivative of AfB_1. The major route of excretion of AfB_1 and its metabolites is the biliary pathway, followed by the urinary pathway. In lactating animals, AfM1 and other metabolites are excreted in the milk (Gratz and Täubel, 2007). This carcinogenic mycotoxin was proven to be related with increased lameness (subclinical mastitis) and impaired fertility (cystic ovaries) (Özsoy *et al.* 2004). The Institute of Applied Research on Cancer has included aflatoxins and AfM_1 as part of group 1 and 2B-carcinogens and possible human carcinogens, respectively (IARC, 2002). European Union directives followed in order to establish limits on the content of AfB_1 in feed and of AfM_1 in foodstuffs respectively, 5 µg/kg and 0.05 µg/kg (European Commission, 2003; European Commission, 2006). In an experiment conducted with both low and highly productive cows, the carry-over rate for aflatoxin B_1 into aflatoxin M_1 ranged between 1.8 and 6.2%, with high-producing animals showing higher levels of transference, independently of AfB1 intake (Veldman *et al.* 1992).

Out of the 43 samples tested for fumonisins, 11.6% presented a positive result. The maximum contamination level found was 989 ppb. FUM has shown to reduce milk production in dairy cattle (Diaz *et al*. 2000).

ZON and/or DON contamination was found in many samples tested for these mycotoxins. From the 191 samples tested approximately 20% and 40% were positive for ZON and DON, respectively. Levels as high as 26,728 µg/kg (ZON) and 1256 µg/kg (DON) were found for these mycotoxins. Studies have been made with both mycotoxins proving their negative impacts in animal production. Several case reports have related ZON to an estrogenic response in ruminants and sometimes included abortions as a symptom. ZON lowered the conception rate of heifers when given orally at a concentration of 250 ppb (Weaver *et al.* 1986). Other cattle responses may include vaginitis, vaginal secretions, poor reproductive performance, and mammary gland enlargement of virgin heifers. Although scientific trials and research do not clearly evidence a cause-effect relationship between DON ingestion and milk production, many producers have observed a correlation between DON in rations and problems with reduced milk production, feed intake and herd health (Kuldau, 2008). According to Wilkinson (2005) DON is the most common mycotoxin in the silage. Trichothecenes cause weight loss, vomiting, severe skin problems and bleeding and may, in some cases, be responsible for the death of animals. Like aflatoxins, they have immune-suppressive properties acting both on the cell immune system and on the number of macrophages, lymphocytes and erythrocytes. T-2 and DON are known to inhibit protein synthesis and cause cell death in various parts of the body (Yiannikouris and Jouany, 2002).

Table 4 Results of silage analyses in 2007 (Acosta Aragón and Rodrigues, 2009)

Sample	Parameter	Mycotoxin	
		DON	ZON
Corn Cob Mix (CCM)	No. tested samples	2	0
	% positive	100	-
	Maximum contamination level (µg/kg)	386	-
	Average positive samples (µg/kg)	229	-
Whole grain corn silage	No. tested samples	5	3
	% positive	40	-
	Maximum contamination level (µg/kg)	961	-
	Average positive samples (µg/kg)	601	-
Whole plant corn silage	No. tested samples	22	8
	% positive	100	100
	Maximum contamination level (µg/kg)	5815	1043
	Average positive samples (µg/kg)	807	168
Crushed grains corn silage	No. tested samples	11	5
	% positive	100	100
	Maximum contamination level (µg/kg)	3472	1050
	Average positive samples (µg/kg)	781	305

Mycotoxin production and occurrence is not restricted to a single location, it is widespread amongst a large variety of environmental conditions. Monitoring the presence of mycotoxins in samples from different regions is a helpful tool that supports this statement. Table 4 gathers data from analyses performed in 2007 in different corn product silages from Austria.

In this assessment, a total of 40 silage samples were analyzed for DON and 16 were analyzed for ZON contamination. Out of these, 92.5% and 81.3% have shown to be contaminated, respectively. The maximum DON content was 5815 µg/kg, found in whole plant corn silage and ZON contamination as high as 1043 µg/kg was found in the same commodity type.

Table 5 displays the results of mycotoxin analyses conducted in United Kingdom for grass and corn silage, as well as for Total Mixed Ration (TMR) containing different types of silages. The tests were performed by ELISA from November 2007 till January 2008.

Although, HPLC is the most accurate method to quantify mycotoxin contamination of feedstuffs, ELISA also represents a useful and inexpensive rapid tool to monitor the presence of these hazardous compounds. As the results in Table 4 show, all samples (n=12) were positive for both T-2 and ZON contamination. In spite of the small population size, these results support the idea that screening is of extreme importance in mycotoxin risk management.

Table 5 Mycotoxin contamination in grass and corn silage and TMR in United Kingdom in 2007 (Acosta Aragón and Rodrigues, 2009)

Type of sample	Parameter	Mycotoxin	
		T 2 toxin	ZON
Grass silage (n=3)	% positive	100	100
	Maximum content level (µg/kg)	17.2	89.5
	Average positive samples (µg/kg)	11.9	61.8
Corn silage (n=4)	% positive	100	100
	Maximum content level (µg/kg)	13	47.5
	Average positive samples (µg/kg)	7.9	37
TMR (n=15)	% positive	100	100
	Maximum content level (µg/kg)	21.0	154.0
	Average positive samples (µg/kg)	17.6	38.9

Avoiding the effects of mycotoxins

The presence of fungi in silages is more likely to occur than their absence. In order to minimize the occurrence of fungi and mycotoxins in the silage, cautious handling of the crops and good ensiling processes should be assured.

Avoiding mycotoxin formation must begin on the field (crop rotation, use of resistant plants, use of fertilizers, biological and chemical control of fungi and adequate plant maturation), has to continue in the silage making process (with proper hygiene, adequate silage additives, compaction, sheeting and storage length) and finalize with the correct management of the open silo (speed of progression in the silo, discard of deficiently preserved silage, additional use of silage additives for stopping the undesirable fermentations). This will increase the probability of producing high quality silage (Acosta Aragón, 2010). Nevertheless, mycotoxins still occur in feedstuffs and in silage specifically as shown in the tables above.

The rumen mycotoxin detoxification capacity is limited to certain mycotoxins and may lead to the production of compounds more toxic than the original molecule (Yiannikouris and Jouany, 2002). Moreover, this capacity is highly dependent on ruminal microbial ecosystem, which is in turn highly sensitive to nutritional changes. Finally, it should also be considered that the rumen is negatively impacted by the occurrence of these toxic compounds showing a decrease in rumen contractions and motility (Cook *et al.* 1986; Froetschel *et al.* 1986), lower digestion of Dry Matter, ADF (Acid Digestible Fiber) and starch (Froetschel *et al.* 1987).

Several physical, chemical and biological methods have been developed to counteract mycotoxins in the feed, preceding animals' ingestion. Physical and chemical methods show many disadvantages, ranging from uncertainty of results to toxicity of by-products formed and high costs and high losses associated with the treatments. Biological methods comprise binding by adsorptive material as well as microbiological inactivation by specific microorganisms or enzymes (Schatzmayr *et al.* 2006). The latter, also referred to as biotransformation, offers a better solution for a wider range of mycotoxins, especially for those which cannot be fully bound by minerals.

Within the complex field of mycotoxins resulting from analytical problems (namely sampling, accuracy of results and occurrence of masked mycotoxins), complexity of mycotoxicoses diagnostic and still lack of information regarding these molecules, amongst other facts, the only certainty is that these compounds impact negatively animals and humans in contact with them. No safe levels can be assured

since when dealing with living beings such as animals many factors have to be taken into account and are impossible to be controlled. Prevention of risks, however, is possible, and should be done not only for silages but for all feedstuffs.

CONCLUSION

There are many types of silage are contaminated by mycotoxins and even the best prevention cannot eradicate these toxins totally. Relatively high quantities of DON, ZON, T–2 toxin and fumonisins have been found in visually moulded silages but also apparently good silages.

The prevention of the mycotoxin contamination must begin in the field in early phenological phases of the crop, however, many factors are out of the control of the farmers (temperature and composition of the gas atmosphere), others are difficult to manage (substrate properties, pH and chemical composition of the soil), as well as biotic factors (insects, vertebrates and microorganisms). The increase in the plant resistance against moulds is a controversial topic nevertheless an effective tool to keep the contaminations at low level.

In addition, a deficient storage can diminish markedly the feed quality. The consequences of mould occurrence are high losses of dry matter and nutrients, fungal growth and mycotoxin production which have negative impacts on animal performance and health. Good ensiling practices and feed out phase management are essential to avoid mould growth and mycotoxin formation in the ensiled material. Two important aspects for controlling the oxygen entrance into the silage and the subsequently mould growth and mycotoxin contamination are compaction and coverage. When the silo is open, the farmer has to guarantee an adequate advance to avoid aerobic instability and secondary mycotoxin contamination.

The detoxification capacity of the rumen is limited, especially in high yielding cows. Physical, chemical and biological methods are already available to counteract mycotoxins in the feed; however, the emphasis must be placed always in the prevention of mycotoxin contamination.

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