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Review

Fungal populations and mycotoxins in silages: From occurrence to analysis

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ABSTRACT

Silage making increased considerably from the 1960s and is the predominant method of forage preservation in temperate areas of the world. Silage is widely used in farms and has a substantial role in animal production systems. Currently, silage quality is evaluated by chemico-fermentative parameters. However, the presence of bacteria, moulds, and/or some of their metabolites, i.e., mycotoxins, must be considered because of their effects on animal production and health. The knowledge of mycotoxin occurrence in animal feed was concentrated primarily on commodities, such as grains and cereals. However, the contribution of silages to total mycotoxin intake could be significant and sometimes greater than that of compound feed in ruminant diet, as forages are the main dry matter component. The silage process is mainly under the control of the farmer. Therefore, large differences in preservation quality can be found, and different fungi found in forage may lead to a varied spectrum of toxins. The evidence regarding fungi and mycotoxins occurring in different silages from different geographical areas, and the fate of fungi and mycotoxins during ensiling, confirm the need to monitor the quality of silage that is fed to animals. Economical and straightforward silage testing is critical to reach a quick and sufficiently accurate diagnosis of silage quality, which allows for "in field" decision making with regard to the acceptability of a given forage for its use as animal feed.

This review describes several topics of interest regarding fungi and mycotoxin contamination in silages, focusing on their occurrence as well as factors affecting their concentrations and distribution at harvest and during ensiling. The impact on sampling and analysis will also be discussed.

Abbreviations: 15-ADON, 15-acetyldeoxynivealenol; 3-ADON, 3-acetyldeoxynivalenol; AFB1, aflatoxin B1; AFLA, aflatoxins; AFM1, aflatoxin M1; a_w, water activity; a-ZOL, a-zearalenol; b-ZOL, b-zearalenol; CE, capillary electrophoresis; CPA, cyclopiazonic acid; CTN, citrinin; Dc-ELISA, direct competitive ELISA; DON, deoxynivalenol; EN, electronic nose; FPI, fluorescence polarization immunoassay; FT-IR/ATR, Fourier Tansform Infrared/Attenuated Total Reflectance; FT-NIR, Fourier Tansform Near Infrared; GT, gliotoxin; HPLC-MS, high performance liquid chromatography-mass spectrometry; HT-2, HT-2 toxin; IR spectrometry, Infrared spectrometry; LC-MS/MS, liquid chromatography-mass/mass spectrometry; LFD, lateral flow device; MAS, 15-monoacetoxyscirpentriol; MI spectroscopy, Mid-infrared spectroscopy; MIP, molecularly imprinted polymers; MOSs, Metal Oxide Semiconductors; MPA, mycophenolic acid; NIV, nivalenol; OTA, ochratoxin A; PAT, patulin; PCR, polymerase chain reaction; ROC, roquefortine C; SCIRP, scirpentriol; SPR, surface plasmon resonance; T-2, T-2 toxin; UHPLC-MS/MS, ultra high performance liquid chromatography-mass/mass spectrometry; ZEA, zearalenone.

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Contents

1. Introduction 2
2. Occurrence of fungi and mycotoxins in field and during ensiling 3
2.1. Pre-harvest events 3
2.2. Post-harvest events 4
3. Occurrence of fungi and mycotoxins in mature silages 6
4. Occurrence and distribution of fungi and mycotoxins within a silo and during feed-out 8
5. Fungi and mycotoxin analytical detection 9
6. Conclusions and future perspectives 12
References 13

1. Introduction

Silage making has increased considerably since the 1960s in temperate areas of the world (Wilkins, 2005) and is a common approach to storing and feeding forage for milk- and meat-producing ruminants. The preservative effect against the growth of detrimental microorganisms, including fungi, is a result of anaerobiosis and acidification of the ensiled forages by lactic acid-producing bacteria. The growth of these bacteria is encouraged by the addition of starter cultures and/or organic acids and by creating an anaerobic microclimate by compression of feed material (McDonald et al., 1991; Scudamore and Livesey, 1998). Forage silage as a source of mycotoxigenic fungi and mycotoxins merits attention (Fink-Gremmels, 2005; Richard et al., 2007; Storm et al., 2008, 2010; Wilkinson, 1999). Mould contamination in silage is associated with reduced palatability, reduced nutritional value and feed intake, animal health problems, decreased productivity and fertility and increased disease susceptibility (Fink-Gremmels, 1999, 2008b; Scudamore and Livesey, 1998). Moulds are able to produce several toxins; multi-mycotoxin contamination is of particular concern due to potential additive or synergistic effects on animals exposed to mouldy silage. These effects range from reduced or refused intake to neurological, estrogenic, hepatotoxic and immunotoxic effects (D'Mello et al., 1999; Fink-Gremmels, 2008b; Scudamore and Livesey, 1998; Wilkinson, 1999). Annually, approximately 25% of crops worldwide are affected by mycotoxins (Fink-Gremmels, 1999; Hussein and Brasel, 2001). Mycotoxins contribute to economic losses due to negative effects on livestock productivity, crop losses and the cost of regulatory programs directed towards mycotoxin analysis (Hussein and Brasel, 2001; Schmale and Munkvold, 2009). Mycotoxins form a distinct group of secondary metabolites produced by certain fungi (including the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Stachyobotris*, and *Cephalosprium*). The true number of mycotoxins in animal feed remains to be determined, as new fungal metabolites are still being discovered and awaiting assessment of their potential and synergistic contribution to diseases in animals (Fink-Gremmels, 2008b).

Rumen microflora can degrade and inactivate mycotoxins; as a result, ruminants are among the least susceptible animal species. However, in a review on the role of mycotoxins in the health and performance of dairy cows, Fink-Gremmels (2008b) noted that the rumen detoxification capacity might be saturable and can vary with changes in diet or the presence of metabolic diseases. Another point of concern is that some mycotoxins have a high carry-over rate from feed to milk, possibly contributing to mycotoxin intake in human populations (Fink-Gremmels, 2008a). Ruminants can be exposed to a broad range of mycotoxins that occur in concentrates, pasture forage and in preserved feedingstuffs, such as silage and hay. Research on mycotoxin occurrence in animal feed has primarily focused on commodities, such as grains and cereals (Bhat et al., 2010; Binder et al., 2007; Placinta et al., 1999; Scudamore et al., 1998; Zinedine et al., 2007). However, mycotoxins found in forage may represent a significantly different spectrum of toxins. Moreover, the contribution of forage to the total dietary intake of mycotoxins may be significant as forages are the main dry-matter component of the ruminant diet. A survey conducted by Driehuis et al. (2008a) estimated the total dietary intake of mycotoxins by dairy cows on 24 farms in the Netherlands. Silage and compound feed were the main components of the diet, representing an average 67% and 23% of the dry matter intake, respectively. The authors found that relative to compound feed, the contribution of silage to total intake of deoxynivalenol (DON) and zearalenone (ZEA) was 3.5 and 2.9 times greater, respectively. Furthermore, probably it can be assumed that mycotoxins levels in silage may exceed the existing regulatory limits under certain circumstances. For example, to avoid the carry-over of aflatoxin B1 (AFB1) to milk as aflatoxin M1 (AFM1) in higher concentrations than 50 μg/kg as EU limits for in AFM_1 raw milk (Commission Regulation (EC) No 1881/2006 of 19 December 2006), the maximum daily intake of AFB1 should not exceed 40 μg/animal/day for a 30 kg milk per day producing cow (Driehuis et al., 2008b). As a consequence, it could be possible to forecast the maximum concentration of mycotoxin acceptable for each kg of silage fed to animals on the basis of other ration ingredients contamination and quantity of silage distributed.

Given the potential for mycotoxin contamination, it is important to obtain information about the type and distribution of mycotoxins in silages. Mycotoxin levels should be monitored in control programs to make a precise evaluation of the quality of the silage being fed to animals.

This review describes several topics of interest regarding fungi and mycotoxin contamination in silages and focuses on their occurrence and factors affecting their concentrations at harvest and during ensiling. In particular, the impact on sampling and analysis will be discussed.