AgriSearch
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AgriSearch Silage Mycotoxin Screening Survey 2024/2025

REPORT

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Summary

AgriSearch carried out a screening study to examine the level of mycotoxins present in grass silages across 13 farms in Northern Ireland in the winter of 2024/25. A total of 43 silage samples were obtained, with samples frozen following collection and subsequently analysed by Queen's University in Spring 2025. The majority of the 43 silages had very low or no mycotoxin present. Where low levels of mycotoxin were detected, these could mainly be attributed to *Fusarium* fungi – associated with soil and dead/dying grass ensiled along with grass. Very low levels of mycotoxins (less than 25 parts per billion (ppb)/kg fresh weight (FW)) related to *Penicillium* (a blue-green powdery mould) were detected in 5 silages, but these are unlikely to have impacted animal performance. One silage had 40 ppb Penicillic acid/kg FW, a borderline level of contamination which may have impacted intake and animal performance. This sample was obtained from a third cut silage of high quality, but which had shown signs of heating at the silo face. A further silage had a relatively high level of mycophenolic acid (MPA 134.1 ppb /kg FW) and this may also have resulted in reduced animal performance. This was a first cut silage ensiled on 10 May following a 24-hour wilting period.

In both silages in which *Penicillium* mycotoxins were detected, slurry had been applied with splashplate slurry spreading equipment at least seven weeks previously. Whilst there was evidence of residual slurry bands on swards in eight of the farms which used LESSE slurry spreading equipment, there was no evidence in this study that this resulted in mycotoxin contamination of silage.

Introduction

Mycotoxins are natural substances produced by moulds and fungi either in the field (eg *Fusarium* sp) or during storage (e.g. *Penicillium* and *Aspergillus* sp) and are widely found in the natural environment. They are invisible, tasteless and toxic (when present in high concentrations), with more than 500 different mycotoxins being identified to date. Most animal feedstuffs are contaminated to some degree. In relation to animal performance, mycotoxins pose significant risks to livestock, including reduced feed intake, immune suppression, and reproductive issues. Mycotoxins can reduce appetite through impaired rumen function, resulting in reduced milk yield, poor milk solids or reduced liveweight gain. Some studies have reported effects on herd fertility with lower conception rates and increased risk of abortion.

There have been reports of increasing incidence of Mycotoxin contamination in grass silages in Northern Ireland. In some extreme cases this has led to antibiotic failures of milk tanks. Negative effects have also been reported on cow health, fertility and production efficiency, and several feed companies are now recommending the addition of 'mycotoxin binders' as dietary additives to counteract these negative effects. These binders are normally based on specific strains of yeast, and are described as broad-spectrum binders that tackle mycotoxins in general, rather than dealing with individual mycotoxins. At present there is a lack of independent evidence as to the extent and causes of mycotoxin contamination of silages.

There are several factors which might contribute to the reports of increasing incidence of mycotoxin contamination in recent years. Factors such as plant stress (ensiling in very dry or very wet conditions), excessive slurry and/or soil contamination of grass swards and poor ensiling technique (inadequate compaction/ delayed and/or poor sealing of silos) have been highlighted. In addition, new methods of slurry spreading involving the use of Low Emission Slurry Spreading Equipment (LESSE) such as trailing hose or trailing hose equipment have been implicated, as these methods concentrate the slurry in a narrow band on the soil surface, and this can contribute to increased slurry contamination of the grass sward, particularly if high solid content slurry is applied to high stubble residues during prolonged dry periods. The residual slurry can provide a substrate for moulds like *Penicillium* to thrive, with the moulds ensiled along with grass. This can result in pockets of contamination within the silo.

In response to the increasing reports of mycotoxins being found in grass silage, AgriSearch commissioned a pilot project to examine the prevalence of mycotoxins in grass silage on farms in Northern Ireland. The study involved 13 farmers from AgriSearch's Beacon Farm and GrassCheck farmer networks.

Study Details

A total of 13 farms were selected from AgriSearch's Beacon Farm and GrassCheck farmer networks. The farmers agreed to provide detailed records of slurry and fertiliser applications, cutting dates, details of silage management and prevailing weather conditions at cutting and harvesting during the 2024 silage season. Farmers provided the following details:

- Fertiliser and slurry applications (including slurry application method)
- Timing of cutting, tedding, raking, harvesting etc
- Harvesting methodology
- Ground conditions & weather
- Additives used
- Silo Management
- Post Harvesting stubble assessment (incl. photos)

Silage samples were then obtained from the farms during feed-out in the winter of 2024/25 by AgriSearch staff. In all cases a representative sample of silage was obtained by sampling across the entire silo face, with a composite sample being obtained from at least 8 -10 locations. Samples were also taken from core sampling of the silos behind the feed face and from the feed passage. Samples were frozen at -20 degrees C within 12 hours of sampling and subsequently thawed and analysed by Queen's University, Belfast. The method employed for the detection and quantification of mycotoxins was based on a modified liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical platform to achieve high-precision detection and quantification. An AB Sciex QTrap 5500+ MS/MS system with electrospray ionization (ESI) was coupled to an ExionLC AD system for chromatographic separation using a C18 column. The method utilized a binary gradient elution and fast polarity switching for efficient detection of antibiotic compounds in both positive and negative modes. Strict instrument settings and compliance with SANTE/11312/2021 guidelines ensured analytical accuracy and reliability, including the use of scheduled multiple reaction monitoring (sMRM) with dual MRM transitions per analyte for robust confirmation.

Results

Silage production A summary of information relating to silage production across the thirteen farms is presented to Table 1.

Table 1 Summary of farmer responses on silage production system

	Yes	No	
Previous issues with mycotoxins	9	4	
Currently using mycotoxin binder	7	6	
Use Total Mixed Ration for feeding	8	5	
Use LESSE for slurry application	9	2	(2 farms used SP and LESSE)
Evidence of slurry lines in sward	8	5	

The majority of farms reported previous issues with mycotoxin contamination, with 7/13 farms using binders during feed-out as a precautionary measure. Slurry application was primarily by LESSE (9/13 farms), with 2 farms using splashplate (SP) spreaders and 2 farms using a combination of LESSE and SP spreaders. First cut slurry application varied from 3 February to 30 March, with the first chemical fertiliser being applied from 22 February to 13 April. First cut harvest date ranged from 25 April to 18 May, with a 24-hour wilt practiced on the majority of farms (9/13) and a 48-hour wilt on 4 farms. Silage was big-baled on 2 farms, with grass ensiled in clamp silos on the remaining 11 farms. The majority of farms (8/13) did not use a silage additive, with 5 farms using silage inoculant additives. Eight farms observed evidence of slurry lines in the sward at harvest which were associated with use of LESSE slurry spreaders, with none of the farms who used SP spreaders observing slurry contamination of the sward at harvest.

Mycotoxin analysis

Analysis of the silages indicated low levels of mycotoxins associated with *Fusarium* species including 15-acetyl-deoxynivalenol (<150 ppb/kg FW), Deoxynivalenol (vomitoxin, <400 ppb/kg FW), Enniatin-A (<4 ppb/kg FW), Enniatin-A1 (<18 ppb/kg FW), Enniatin-B (<180 ppb/kg FW), Enniatin-B1 (<60 ppb/kg FW), Zearalenone (<450 ppb/kg FW) and Nivalenol (< 140 ppb/kg FW). *Fusarium* species are fungi that actively grow on forage in the field and are most often associated with dead and dying plant material. They are normally brought into the silage with the forage at harvesting and are generally not associated with mouldy silage. Levels

detected in these silages are low and therefore are unlikely to have caused animal health or production issues.

Additional analyses were undertaken to determine the levels of *Penicillium* mycotoxins – as these have been linked to milk tank antibiotic failures and impaired animal performance. *Penicillium* moulds are normally associated with storage fungi and have been linked with visible signs of moulding in silage at feed-out. No detectable levels of the mycotoxins Citrinin and Cyclopiazonic acid were found.

Very low levels of Mycophenolic Acid (MPA < 8.4 ppb/kg FW) were detected in 42/43 silages, although one sample (Silage 7) had a higher level (134 ppb/kg FW). This silage was a first cut silage, cut on 9 May and ensiled after a 24-hour wilting period. Slurry had been applied to the sward using SP spreading equipment on 20 February (11 weeks prior to harvest) and no slurry was detected in the sward at harvest. MPA is a harmful mycotoxin produced by *Penicillium roqueforti* mould, often found in spoiled silage, especially where air has entered, causing overheating and increased pH, allowing growth of fungi like *Penicillium*. Low levels of Patulin, Penicillic Acid and Roquefortine C were observed in 7 silages as shown in Table 2. Silage 2 had a higher level of Penicillic acid (40 ppb/kg FW) which is a border line level of contamination which may reduce animal performance. This silage was a third cut silage, cut on 16 August and ensiled within 12 hours of cutting. Slurry had been applied to the sward using SP spreading equipment on 26 June (seven weeks previously) and no slurry was detected in the sward at harvest. The silage had visibly heated at the silo face, but was of high quality with 219 g/kg DM, 12.1 MJ ME/kg DM, pH 3.5 and 120 g crude protein/kg DM.

Table 2 Results of analysis of silages with detectable levels of mycotoxin (all values are presented as parts per billion (ppb) per kg silage fresh weight

Silage Number	Patulin	Penicillic Acid	Roquefortine C	Mycophenolic Acid
1	0	24.0	0	< 9.0
2	0	**40.0**	1.9	< 9.0
3	0	6.7	0	< 9.0
4	0	0.9	0	< 9.0
5	22.5	0	0	< 9.0
6	0	8.3	0	< 9.0
7	12.9	0	6.7	**134.1**

Note no Penicillium mycotoxins were detected in the remaining 36 silage samples.

Conclusions

It is important to note at the outset that the current screening study was relatively small scale involving 13 farms, and undertaken over one silage season. Levels of mycotoxins were relatively low or non-detectable across 41 of the 43 silages and were unlikely to have affected animal performance. Two of the silages had detectable levels of *Penicillium* mycotoxins, with one containing a low level of Mycophenolic Acid (134 ppb/kg FW) and a second containing a borderline level of Penicillic acid (40 ppb/kg FW). In both cases, slurry had been applied with splashplate slurry spreading equipment at least seven weeks previously. Whilst there was evidence of residual slurry bands in eight of the farms which used LESSE slurry spreading equipment, there was no evidence in this study that this resulted in mycotoxin contamination of silage.

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