Toxicity, occurrence and negative effects of PR toxin – the hidden enemy

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The PR toxin is a secondary metabolite of Penicillium roqueforti. It has long been reported that poorly ensiled crops, which promote the growth of Penicillium moulds, pose a serious threat to the health and productivity of animals and result in significant economic losses.

This article reviews the present knowledge on the toxicity, occurrence and negative effects of PR toxin in animals.

Ensiling or fermenting forages with lactic acid producing bacteria is a relatively simple yet effective way of preserving forage for future use by livestock.

One of the spoilage fungi most commonly found in silages as well as other habitats with limited oxygen is Penicillium roqueforti, and under such improper ensiling conditions, spoilage by Penicillium as well as Aspergillus moulds that were present in the feedstuff at harvest is likely to occur accompanied with some degree of mycotoxin contamination.

One of the mycotoxins of concern that is produced by Penicillium roqueforti is PR toxin. The effects of long term PR toxin exposure are a subject of interest since ruminant diets frequently contain a high proportion of forages (dairy cows eating 15±kg/dry matter/day) such as grass or maize silage, hay and straw. High producing livestock, especially, can be subject to subacute symptoms of PR toxin mycotoxicosis.

Besides PR toxin, other toxins such as roquefortine C, patulin and mycophenolic acid can be produced by this fungus.

Auerbach et al. (1998) found P. roqueforti in 89% of visibly mouldy samples and in 85% of visibly unmouldy samples rendering visible inspection essentially useless in identifying Penicillium mould contamination of forages.

Chemical structure

The PR toxin molecule contains several functional groups including an aldehyde, an acetoxy, and an α, β-unsaturated ketone group, in addition to two stable epoxide rings (Fig. 1).

The aldehyde group appears to play an important role in biological activity. The biosynthesis of PR toxin has been studied with both 14C- and 13C-labelled precursors and indicates that PR toxin is formed via an isoprene biosynthetic pathway.

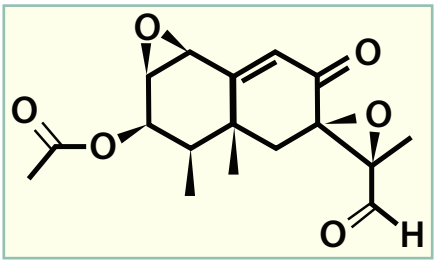

Fig. 1. Chemical structure of PR toxin.

Eremofortin C (EC) (another secondary metabolite) and PR toxin are closely related to each other and differ only by a hydroxyl functional group in EC and an aldehyde functional group in PR toxin at the C-12 position responsible for the conversion of EC into PR toxin. In contrast to PR toxin, EC does not show significant toxicity in animals.

Toxicity

PR toxin has been shown to be lethal to animals and exhibits a broad spectrum of biochemical activities, which cause toxicoses in animals.

Up to now, studies with laboratory animals report that PR toxin is considered to be the most toxic of the P. roqueforti mycotoxins. This toxin is lethal to rats and mice by either oral or intraperitoneal (ip) administration. LD50 values in mice ranged from 1.0-5.8 mg/kg (ip) and 58-100mg/kg/BW orally.

PR toxin causes degenerative changes in livers and kidneys of rats. Wei and Liu (1978) verified inhibition of in vivo protein synthesis in rat liver, probably because the toxin prevents the initiation and elongation steps of transcription. In addition to the inhibitory effects on proteins, this mycotoxin has also revealed strong inhibitory effects on ribonucleic acid and deoxyribonucleic acid biosynthesis of Ehrlich ascites tumour cells.

Carcinogenic activity has also been shown to result from PR toxin exposure. Rats fed PR toxin developed adenocarcinoma, squamous epithelioma and a uterine sarcoma, which were shown histologically. Tests with Salmonella typhimurium, Saccharomyces cerevisiae and Neurospra crassa also showed mutagenicity from PR toxin exposure.

Toxic effects in mice and rats have included abdominal writhing, decreased motor activity and respiratory rate, weakened hind legs and ataxia. In mice, rats and cats PR toxin given ip caused ascites fluid and oedema in the scrotum and lungs.

Intravenous (iv) injection caused oedema in the lungs, giving rise to a large volume of pleural and pericardial fluid. Injecting rats with LD50 doses (11.6mg/kg, ip and 8.2mg/kg, iv), resulted in an increase in the water content of the lungs but a decrease in the skin.

Still et al. (1972) and Chen et al. (1982) conclude that PR toxin produced acute toxic effects in animals via an increase of capillary permeability and a direct damage to the lungs, heart, liver and kidneys.

These researchers also suspected PR toxin was a vector in a case study where abortion and retained placenta occurred.

Little information, some of which is controversial, has been reported about PR toxicity in dairy cows.

Vesely et al. (1981) fed dairy cows maize silages infected with Penicillium roqueforti and reported the animals had loss of appetite, cessation of rumen activity, and gastroenteritis.

Scudamore and Livesey (1998) reported that cows aborted their first calves in the seventh and eighth month of pregnancy, but they cautioned about solely attributing these symptoms to PR toxin since Penicillium roqueforti is known to be capable of forming a number of other toxic metabolites.

More recently, Nielsen et al. (2006) observed that cattle consuming Penicillium roqueforti contaminated feed had variable toxic outcomes with some animals exhibiting severe symptoms including haemorrhage and death.

Current data suggest that roquefortine C is not a very toxic mycotoxin in animals. This and also the fact that roquefortine C is produced over a broad range of environmental conditions lead Nielsen et al. (2006)

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to hypothesise that the seemingly well known acute toxicities associated with P. roqueforti contamination were due to PR toxin or compounds other than roquefortine C. Despite the symptoms reported by these authors, Sumarah et al. (2005) stated that PR toxin does not seem sufficiently toxic on its own to explain symptoms such as general ill-thrift, abortions and more severe toxic signs.

Indeed, there is little information on the toxic effects of PR toxin in dairy cows and therefore more research is needed regarding whether PR toxin does in fact cause problems in dairy cows and at which contamination levels.

This need for more research is highlighted in a recently published review about mycotoxins in silages in which Storm et al. (2008) state that the toxic effects of PR toxin in ruminants are still unknown.

Occurrence of PR toxin

O'Brien et al. (2006) analysed secondary metabolites produced by Penicillium paneum and Penicillium roqueforti from baled grass silage.

A total of 157 isolates were investigated, comprising 78 P. paneum and 79 P. roqueforti isolates randomly selected from more than 900 colonies cultured from the silage bales. Roquefortine C, marcfortine A, and andrastin A were consistently produced, whereas PR toxin and patulin were not.

These findings mostly agreed with the literature as noted in Table 1.

Roquefortine C and mycophenolic acid are the two most frequently detected mycotoxins produced by the P. roqueforti group in silages (see photograph below), whereas PR toxin and patulin have been detected only occasionally owing to their unstable nature in this matrix.

Penicillium roqueforti was the predominant fungus found during a study of spoiled maize silage carried out in the Netherlands between 1986-1990. No PR toxin was detected although lumps of infected silage contained several unidentified fungal metabolites.

In contrast, Yu et al. (1999) analysed 63 samples (25 hay and 38 other feedstuffs, including corn silage and mixed feed) and showed that the frequency of PR toxin in these samples was 76% with an average contamination of 130ppb.

Therefore, in light of results such as these PR toxin has been suggested as the responsible agent for problems resulting from feeding mouldy corn silage. Surveys of grass and corn silage in Europe reported an occurrence of P. roqueforti in up to 40% of samples, which was associated with cattle disorders, but the occurrence or levels of PR toxin was not reported.

P. roqueforti	P. paneum	P. cameum
Roquefortine C	Roquefortine C	Roquefortine C
Roquefortine D	Marcofortines A-C as P. roqueforti	Festuclavine as P. crustosum
Roquefortines A and B	Patulin	Roquefortines A and B as P. crustosum
PR-toxin	Orsellinic acid as P. roqueforti	Mycophenolic acid
Eremofortins A-E	Botryodiplodin as P. roqueforti	Patulin as P. roqueforti and as P. roqueforti var. Cameum
PR-imine		Penicillic acid as P. suavolens and as P. roqueforti
PR-amide		Cycoplastic acid
Mycophenolic acid		Penitrem A

Table 1. Mycotoxins and other secondary metabolites from Penicilliums (Nielsen et al., 2006).

Stability of PR toxin

Penicillium roqueforti requires amino nitrogen for metabolite formation, and the toxins it produces can be formed under conditions of low oxygen (20-30% saturation).

Even though PR toxin is of greater toxicological concern based on studies with laboratory animals, the toxin is known to be unstable and to occur bound to amino acids, amines and NH3. In another study, Chang et al. (1993), reported that PR toxin was transformed into PR-imine when PR toxin was incubated with the culture medium during 37 days, and PR toxin can also be degraded into PR acid. Thus, these researchers proposed that PR toxin is degraded into PR-imine and PR-amide in the culture medium of P. roqueforti.

Müller and Amend (1997) inoculated maize silage with Penicillium roqueforti strains which were capable of forming the mycotoxins mycophenolic acid (MPA), patulin (PAT), penicillic acid (PA) or PR toxin (PRT).

MPA, PAT, PA and PRT levels above the detection limit were measured for the first time at 36, 22-27, 13, and 49 days of incubation, respectively.As storage times increased toxin concentrations decreased to a low or non-detectable level.

Therefore, the researchers, Müller and Amend (1997), concluded that the probability of detecting these mycotoxins in P. roqueforti moulded maize silage is low under practical conditions of agriculture during the growth phase of this fungus and again after prolonged storage.

Although the knowledge about PR toxin is still low, it is hypothesised that the reaction of this toxin with silage contents (for example NH3) may explain the low toxicity. This could also account for these compounds quickly becoming undetectable in the occurring substrate.

Conclusions

Investigations with laboratory animals regarding the toxicity of PR toxin indicated that this mycotoxin is considered the most toxic amongst P. roqueforti toxins. However, the effects of PR toxins on dairy cows are still largely unknown as very few studies with PR toxin and dairy cows are available.

Additionally, due to fact that PR toxin is not stable during storage and may react with silage constituents, the toxicity may be very low except when recently formed concentrations are high.

The detection of these toxins in silages under practical conditions is also difficult, again possibly due to their short life and the apparent instability. On a practical point of view, the usual presence of more than one mycotoxin in silages may be a more accurate explanation for the problems often found in dairy cattle rather than the presence of PR toxin alone. ■

References are available from the authors on request

Penicillium roqueforti in maize silage.