Rejection by pigs of mouldy grain containing deoxynivalenol

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SUMMARY: Weaner pigs on a farm near Beaudesert in south eastern Queensland refused to eat feed comprised largely of wheat and barley. Older pigs consumed small amounts and some prepubertal gilt's subsequently displayed enlarged and reddened vulvas. Wheat, barley and triticale were grown on the farm during 1983, which was unusually and persistently wet. The wheat and triticale were harvested and stored for about 3 weeks with moisture contents above 14% before being fed. Samples of the wheat and triticale contained pale pink grains, which can indicate infection by the fungus Fusarium graminearum Schew. On analysis 2 mycotoxins known to be produced by F. graminearum were detected, deoxynivalenol (vomitoxin) which causes feed refusal and vomiting, and zearalenone which causes oestrogenic effects. Concentrations of deoxynivalenol in the wheat, triticale and barley were 34, 10, and <0.1 mg/kg respectively. Concentrations of zearalenone were 6.2, 2.8 and 0.1 mg/kg respectively. Subsequently, F. graminearum was isolated from grains and crop residues. Although the wet weather contributed to F. graminearum infection of the crops before harvest, most of the toxins probably developed during storage.

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Introduction

Grains fed to livestock in Australia occasionally suffer from weather damage and mould growth, which may be accompanied by mycotoxin contamination. Mycotoxins implicated in reduced production in countries with a temperate climate are the aflatoxins and ochratoxins produced by Aspergillus spp and Penicillium spp, as well as zearalenone and deoxynivalenol (vomitoxin), produced by Fusarium spp. Pigs appear to be more susceptible to the effects of these toxins than either poultry or cattle.

Reports from this Institute have described cases of intoxication of pigs by aflatoxin (Ketterer et al 1982) and zearalenone (Blaney et al 1984a). We now describe cases in which pigs refused to consume diets based on weather-damaged grains in which we identified deoxynivalenol and zearalenone.

Analytical Methods

Mycotoxin Assay

Samples were hammer milled and then assayed for aflatoxins B1, B2, G1 and G2, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin by a thin-layer-chromatographic screening method (Blaney et al 1984b).

Zearalenone was measured by the high performance liquid chromatographic method described by Blaney et al (1984a).

Deoxynivalenol, which is not easily detected in the screening assay, was extracted by the method of Scott et al (1981). The resultant extracts were then dissolved in 0.5 ml of chloroform, and applied to short columns of silica-gel §. The first 14 ml of chloroform were discarded, and the deoxynivalenol was eluted with 10 ml of methanol:chloroform (5:95). After evaporation of solvent, 100 μl of N-trimethylsilyl imidazole were added, and the mixture was allowed to stand for 1 h at room temperature (25°C). Deoxynivalenol was identified using a Finnigan 1020 B gas chromatograph-mass spectrometer, fitted with a 30 m, SE-54, fused silica capillary column. Single ion monitoring at the molecular ion mass of 512 confirmed the presence of the trimethylsilyl derivative of deoxynivalenol. Measurement was performed on the reconstructed ion chromatogram by comparison of peak areas with that of standard deoxynivalenol.

Fungal Isolation and Identification

Fungal isolates were obtained from perithecia (the structure from which ascospores are released) on stubble, grain and glume (husk) samples. These produced perithecia of Gibberella zeae (Schew) Petch (the perithecial state of Fusarium graminearum Schew) when cultured on carnation leaf agar incubated under ultra violet light (Tio et al 1977).

History

Feed rejection by pigs was reported from 2 farms near Beaudesert in south eastern Queensland during January 1984. In one case the feed, described as wheat containing pink grains, was subsequently fed to dairy cows. However, efforts to obtain samples and more detailed information were unsuccessful. In the case investigated, various crops were grown on the farm (including maize in summer) to provide feed for a sow pig piggery and a few chickens. Most of 1983 was unusually and persistently wet (Figure 1), and in late December crops of wheat, triticale and barley were harvested between periods of heavy rainfall. The grains were then stored in silos without drying. Three weeks later the wheat and barley were used to prepare diets for weaner, grower and breeder pigs. Concentrations of wheat in these diets were 60%, 40% and 35% respectively. Barley concentrations were 24%, 45% and 52% respectively.

When the diets were offered to the pigs the weaners completely rejected their feed. Feed intakes by grower pigs seemed to be reduced but sow's were less affected. Molasses was added to the weaner diet but this was ineffective in promoting feed consumption. After 3 to 4 days some of the young gilts weighing 30 to 50 kg were observed with red and swollen vulvas. Consumption of the weaner diet was improved when it was diluted with an equal amount of proprietary feed. About 5 days after the diets were first offered, the weaners and growers had lost weight and the farm-mixed weaner and grower diets were withdrawn. The breeder diet was replaced several weeks later, when consumption had declined sufficiently to cause loss of weight during lactation.

Subsequently, new diets were prepared from the triticale (35 to 40%) and barley (45 to 52%), and these seemed to

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§ Sep-pak® cartridges, Waters Associated Inc, Milford, MA0757, United States of America

around the nodes, particularly on the upper part of the stalk.

Samples of the stored grains were first separated into thrashed
grain, unthrashed grain and trash (1 to 7% of total). There
were numerous perithecia on the glumes surrounding the
unthrashed grain and on loose chaff in the grain. The
thrashed grain was further separated into grain on which no
mycelia and perithecia were visible, grain with visible pink-
red mycelia but no perithecia, and grain with perithecia. The
results are given in Table 2.

Discussion

Consumption of grain moulded with F. graminearum is known to produce 2 different syndromes. The first is an
oestrogenic syndrome primarily due to zearalenone. The
second involves refusal by pigs to consume the mouldy grain.
Vomiting may occur if sufficient is consumed. Deoxynivalenol
is a major contributing factor to this syndrome (Vesonder
et al. 1976). Poultry and cattle are much less susceptible than
pigs to deoxynivalenol and zearalenone.

It was reported by Yeung et al. (1983) that dietary
deoxynivalenol concentrations of 20 mg/kg supplied to pigs
in mouldy maize caused vomiting, 12 mg/kg caused almost
clearance of feed refusal and 1.3 mg/kg significantly depressed
feed intakes and rates of gain. Vomiting was not observed in
the Beaudesert case, but the pigs were not closely observed
when the diets were first offered. The concentrations of
deoxynivalenol in the diets (Table 1) were sufficient to
account for the observed feed refusal. Higher concentrations
of toxins were present in the grain samples than are accounted
for in the mixed diets, but the grain samples were "grab"
samples, and not representative of the bulk in the silos.

Although zearalenone was also present in the wheat, oestro-
genetic effects were small compared to those described by
Beyne et al (1984a), since insufficient feed was consumed.

Fungal Infection, Growth and Mycotoxin Production

F. graminearum Grp 2 (Francis and Burgess 1977) causes
cab and stalk rot of maize and head scab of wheat, barley
and rye. Head scab on the winter cereals is rare in
Australia except in warm, humid regions where maize is
grown during the summer and the fungus survives on crop
debris such as maize stalks and cobs. Dispersal of fungal inoculum to growing crops may occur by rain splashdown or
wind-driven rain, but insects or birds may also act as vectors
(Sutton 1982). The crops are most susceptible to fungal
invasion during anthesis (flowering) and grain formation.
Persistent wetness at this stage favours scab development.
Epidemics of head scab of wheat in Canada have developed
within single seasons (Sutton 1982). In head scab develop-
ment, there is little opportunity for transfer of the fungus
between plants. Disease severity is directly proportional to
the amount of inoculum present and therefore to the amount of
crop debris infected with F. graminearum.

Several factors probably contributed to head scab in the
grains in the Beaudesert area. Firstly, there were maize crop
residues on the soil. Secondly, the autumn and winter of
1983 were unusually wet and persistently wet (Figure 1), allowing
build-up of inoculum on debris. Thirdly, anthesis and grain
favourability of the crop occurred in October which had a
large number of rainy days. Coupled with heavy rains and
uncontrolled weed growth, this provided continuous warm
and humid conditions during this month. Perithecia of F.

TABLE 1
Mycotoxin concentrations in grains and silos

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zearalenone (mg/kg)</th>
<th>Deoxynivalenol (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>4.3</td>
<td>34.4</td>
</tr>
<tr>
<td>Barley</td>
<td>0.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Triticale</td>
<td>2.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Winter</td>
<td>1.7</td>
<td>17.3</td>
</tr>
<tr>
<td>Grower</td>
<td>1.7</td>
<td>17.3</td>
</tr>
<tr>
<td>Summer</td>
<td>1.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Not detected, <0.1 mg/kg
granularum detected on the grains in Table 2) probably developed on the crop before harvest since perithecial development is stimulated by light.

Once the grain was infected, other factors contributed to mould growth and mycoconia accumulation. The lighter discoloured grain and glumes, which would normally be blown out of the harvester, remained with the wheat (Table 2) because thrashing plates were missing from the harvester and because the grain was harvested while wet, between rain showers. The wheat and triticale were then stored with moisture contents above 12·9%, which are unsuitable for these grains. The relative proportions of wheat, triticale and barley with visible pink-red mycelia but no perithecia (Table 2), possibly reflect the degree of mould growth during storage of the grains, and roughly parallel the quantities of mycoconia detected.

Factors contributing to toxin production by F. graminearum are complex. Relative concentrations of racemic, deoxynivalenol and possibly other toxins may be influenced by the particular isolate of F. graminearum, nature of the substrate, temperature, moisture concentration and gaseous environment (Greenough et al 1983). In northern Queensland, F. graminearum in maize appears to be endemic (Blaney et al 1984b). However, although oesophageal effects have been reported in pigs fed maize containing zearalenone (Blaney et al 1984a), there have been no reports of feed rejection by pigs in the region.

We consider that the conditions leading to these cases were unusual and unlikely to be repeated frequently in Australia. However, in the event of increased F. graminearum infection of crops due to unusual weather, mycoconia concentrations may be minimised by ensuring that affected grains are dried before storage. Affected grain may be fed to chickens or cattle, which are less susceptible than pigs to deoxynivalenol and zearalenone. Low concentrations of affected grain may be used in the diets of pigs, although feed makers could still be below optimum and the economics of this should be carefully assessed.

Acknowledgments

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References


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SHORT CONTRIBUTIONS

Geelikkop in goats

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Geelikkop is a haemoparasitic, photosensitive disease which causes severe economic loss among sheep and goats in the Republic of South Africa (Quin and Rimmington 1935, Clare 1935; Brown 1966; van Tonder et al 1972; Kellerman et al 1990). Recently, 2 outbreaks of the disease involving sheep in New South Wales have been described (Goldsmith et al 1984). Field and experimental evidence indicates that geelikkop is caused by an underidentified toxin in the annual herb Tribulus terrestris, acting in concert with sporidemin produced by the saprophytic fungus, Pithomyces chartarum (Kellerman et al 1986). As geelikkop has not been recorded previously in Australian goats, we would like to document its occurrence in a herd from the central western plains of New South Wales.

The disease occurred in a small Aangora stud during early autumn 1983. Affected animals were detected in a group of 35 six-month-old, female kids which were grazing, in rotation, 2 paddocks, each of 1 ha. The paddocks were sprayed irrigated every 2 or 3 days. The pasture was dominated by a prolific growth of T. terrestris, but also contained subtropical clover (Trifolium subterraneum) hay, lucerne (Medicago

saize) and mature rye grass (Lolium perenne) and barley grass (Hordeum leporinum). Six of the 35 kids were clinically affected and of these, 2 succumbed, giving morbidity and case fatality rates of 17% and 31%, respectively. Following diagnosis of photoreactivation for the remainder of the mob was removed from the T. terrestris and no further cases occurred.

Seeding of shade and photophobes were the first clinical signs observed. Examination of the affected animals revealed subcutaneous oedema of the ears, eyelids, nose and submandibular region. Where the resultant swelling was most severe, a yellow serous fluid exuded from the surface of the skin. Affected goats rapidly lost weight and became dehydrated.

Treatment was initiated by moving the 6 affected kids to a darkened shed. Each animal was then given 2 ml of dexamethasone* intravenously and 1 ml of each of tripterygium hydrochloride, B complex vitamincs and folic acid in mg. The treatments were repeated every 3 to 5 days. Two kids died following the course of treatment. The remaining 4 were confined indoors for 10 days and were force-fed leaves of loquat (Eriobotrya japonica) to stimulate appetite and resumption. At the end of this period the subcutaneous oedema had completely subsided and the kids appeared bright and alert. Ten months after the initial occurrence of the disease these animals remained clinically normal, although one was much smaller than its unaffected twin.

* Dexamethasone, products, Richmond, New South Wales
† Tripterygium, Ciba-Geigy Australia Ltd, Lane Cove, New South Wales
‡ Folic acid, Multivet, VR Syndics, Thronleigh, New South Wales
§ Finadene Solution, Holted Agencies Pty Ltd, Boronia, Victoria