Field cases of aflatoxicosis in pigs

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SUMMARY: Five cases of aflatoxicosis in pigs in southern Queensland are described. One peracute case where aflatoxin concentrations of up to 5000μg aflatoxin B_1/kg were demonstrated in stomach contents was presumed to be caused by consumption of mouldy bread. High levels of toxins were also present in the livers. Two cases of acute toxicity were caused by feeding mouldy peanut screenings containing 22000μg aflatoxin B_1/kg. One case of subacute and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 μg/kg). Peracute toxicity caused collapse and deaths within several hours, acute toxicity caused deaths within 12 h and with subacute toxicity deaths occured after 3 weeks on a toxic ration. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occured with peracute toxicity and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.

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Introduction

The first report of toxicity in pigs, which in retrospect was probably due to aflatoxins, described acute and subacute disease in the United States of America in pigs grazing mouldy corn in the field (Sippel *et al* 1953). The acute disease was reproduced experimentally by dosing pigs for several days with corn on which cultures of *Aspergillus flavus* were grown (Burnside *et al* 1957). A more chronic syndrome occured in weaner pigs fed rations containing imported peanut meal in England (Loosmore and Harding 1961). Subsequently aflatoxins were extracted from these imported peanut meals (Sargeant *et al* 1961).

Reports of aflatoxicosis in Australia have described acute disease in poultry fed imported peanut meal (Gardiner and Oldroyd 1965; Hart 1965) acute disease in dogs fed mouldy bread (Ketterer *et al* 1975) and acute disease in calves fed peanut hay (McKenzie *et al* 1981). A case of abortions in pigs has been attributed to feeding mouldy sorghum grain (Connole and Hill 1970).

In this paper 5 field cases of aflatoxicosis in pigs caused by ingestion of mouldy bread, peanut screenings or sorghum grain are presented. They ranged in severity from peracute to chronic and the lesions relating to each level of intoxication are described and compared.

Materials and Methods

The cases occurred during the period May 1980 to June 1981 and were from farms in the Brisbane (case 1), Burnett (cases 2, 3 and 4) and Darling Downs (case 5) areas in southern Queensland.

Pathology

Tissues for histological examination were fixed in 10% formalin, embedded in paraplast and cut at 5 μm. Sections were stained with haematoxylin and eosin and Perl's Prussian Blue.

Analytical Procedures

Aflatoxin analyses were performed using the following methods. In cases 2, 3, 4 and 5, feed samples were analysed by the method of Blaney *et al* (1979) which involves aqueous acetone extraction, chloroform partition and visual estimation under ultra-violet illumination following two-dimensional, thin layer chromatographic separation.

The livers and stomach contents from case 1 were extracted by the method of Trucksess and Stoloff (1979) which is similar to that of Blaney *et al* (1979), but gives slightly better recoveries of aflatoxin M_1. These extracts were then purified further by a Sep-pak* cleanup (Thean *et al* 1980) and analysed by reversed phase high performance liquid chromatography. The instrument used was a Spectra-Physics 8000B with F.S. 970 fluorescence detector, and 10μl injection loop. The column was a Brownlee Labs. 25 cm R.P.-18 column operated at 35°C. The mobile phase was a mixture of methanol (20%), acetonitrile (20%) and water (60%) with a flow rate of 2 ml/min. The detector was fitted with a 418 nm emission filter, with excitation at 365 nm. Eluate from the Sep-pak was evaporated to dryness, then dissolved in 500 μl acetonitrile, and 10μl was injected into the chromatograph. This procedure allowed quantification of aflatoxins B_2, G_2 and B_{2a}, after comparison with peak areas of standards. The remaining acetonitrile was evaporated, 20 μl of trifluoroacetic acid was added, and the mixture was then allowed to stand for 15 min. Next, 100 μl of hexane was added followed by a mixture of water (90%) and acetonitrile (10%), so that the volume of the aqueous layer was the same as that of the acetonitrile before evaporation. Injection of 10 μl of the aqueous layer allowed quantification of aflatoxins B_1 (as B_{2a}), G_1 (as G_{2a}) and M_1 (as M_{2a}). This procedure is based on that of Beebe and Takahashi (1980).

Ochratoxin A was detected using a general screening method for a number of mycotoxins and was quantified by both thin layer chromatography and high performance liquid chromatography (C. J. Moore, unpublished data).

Results

Case 1

History and Clinical Signs

The outbreak of toxicity occured on a licensed swill feeding farm in a group of 380 pigs consisting of 150 adult sows, 200 baconers and about 30 porkers. Feed consisted of dry rendered hospital food scraps, stale bread returns from a bakery and vegetable scraps. The pigs were housed in a dirt yard and fed in an adjacent pen on a concrete floor which had space for about 100 pigs. A single early morning feed which was consumed in half an hour was the cause of

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toxicity. Fifty pigs died within 2h, 20 further pigs died within 24h of feeding and 10 pigs died over the following 3 days. Total mortality was 80 pigs, consisting of 70 baconers and adult sows and 10 porkers. The signs seen were frothing and bloody discharge from nose and mouth, coarse tremor and staggering followed by collapse and death as early as 10 min after onset of signs. Depression, vomiting and diarrohea were seen in less acutely affected animals and there was evidence that some abortions occurred.

Pathology

Six pigs (2 mature sows, 2 baconers and 2 porkers) were autopsied 30 h after injestion of the toxic feed. In the 4 older animals large volumes of dark blood stained fluid were present in thoracic and abdominal cavities. There was congestion of the submandibular and pharyngeal areas, extensive haemorrhage in diaphragm muscle and copious haemorrhage into the lumen of the lower half of the small intestine, the caecum and the upper part of the colon. Congestion and oedema of lungs and congestion of the liver with marked oedema of the gall bladder wall were constant findings. Stomachs were dilated with dirty grey semifluid material in which vegetable scraps were readily identified as well as large quantities of sand. Marked haemorrhage of a gastric lymph node was present in one pig and congestion and enlargement of the spleen was seen in another.

Both of the smaller pigs had a firm congested liver and oedema of the gall bladder wall as well as blood in the caecum and anterior colon.

Histological examination of livers of all pigs revealed extensive acute centrilobular necrosis and haemorrhage with a few surviving hepatocytes in the periphery (Figure 1). Extensive subserosal oedema was seen in the gall bladders and there was pulmonary congestion and oedema. No significant changes were seen in kidney, small and large intestine, heart or brain sections.

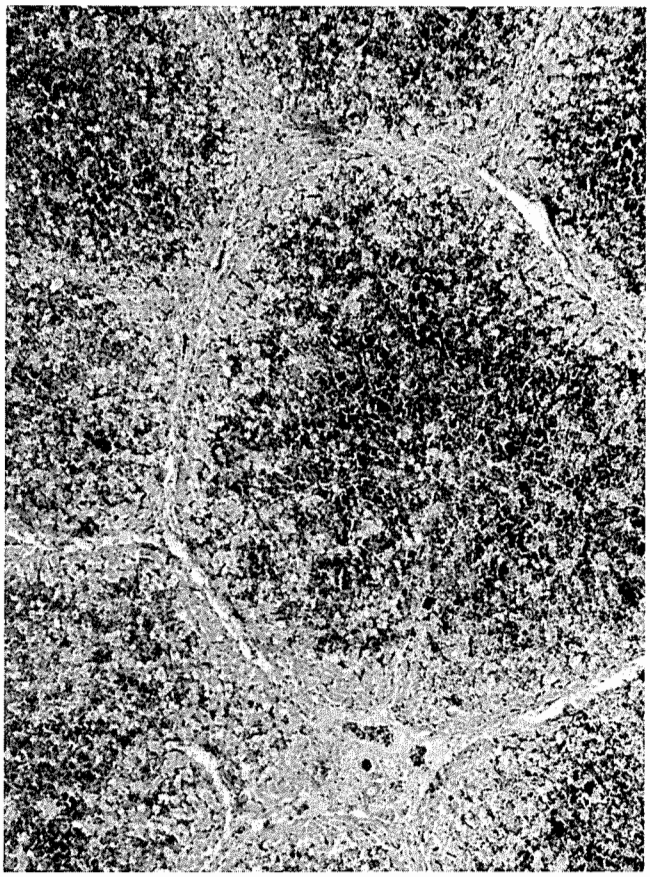

Figure 1. Hepatic centrilobular necrosis and haemorrhage — case 1. Haemotoxylin and eosin X50.

Chemical Analyses

No bread or other feed material was available for analysis. The stomach contents and livers from 5 pigs, together with the liver from a sixth pig were stored at 4°C for 2 weeks prior to analysis for aflatoxins and metabolities of aflatoxins. The proportion of dry organic matter in the samples of stomach content was determined as the loss during ashing of dried portions of the contents. This ranged from 9 to 11% in the 5 samples. The results of aflatoxin analyses expressed on an "as is" basis are shown in Table 1.

TABLE 1

Aflatoxin concentrations (μg/kg) in tissues and feed

Sample type	Sample No.	Aflatoxins B_1	B_2	G_1	G_2	M_1	B_{2a}
Case 1	1	1330	90	†	†	†	15
Stomach	2	670	43	†	†	†	5
contents	3	1200	152	†	†	†	15
	4	2000	200	†	†	†	28
	5	5000	470	†	†	†	50
Liver	1	2.0	0.5	†	†	5.8	†
	2	4.1	1.3	†	†	13.4	†
	3	1.4	0.5	†	†	5.0	†
	4	30.0	5.0	†	†	14.0	†
	5	8.0	1.6	†	†	7.5	†
	6	4.2	1.2	†	†	4.5	†
Case 2	1	20000	2000	12000	1000	*	*
Peanut	2	6000	400	2000	200	*	*
screenings	3	24000	2000	16000	2000	*	*
Case 3	1	10000	500	10000	1000	*	*
Peanut	2	20000	500	20000	500	*	*
screenings							
Case 4		3000	395	5260	900	*	*
Sorghum grain							
Case 5	1	80	11	†	†	*	*
Sorghum	2	250	30	†	†	*	*
grain							

* Not analysed

† Not detected (<5 G_1, G_2, <15M_1 for stomach contents; <0.1 G, G_2, B_{2a} for liver; <1 G_1, G_2 for sorghum grain)

Case 2

History and Clinical Signs

A group of 8 large white sows in a pen and another group of 8 sows and one boar in a small paddock were fed a proprietary ration. One afternoon both groups were given a single feed of mouldy peanut screenings and this caused acute toxicity with signs of severe depression, vomiting, abortion and deaths. A total of 9 pigs died; 2 died within 12 h, another 6 died in the next 12 h and one died a week after ingesting the toxic material.

Pathology

No material was available for pathological examination.

Chemical Analyses

Three samples of feed (each of one kg) were examined and found to contain a mixture of broken and whole peanut kernels, shells, peanut stubble and soil. Aflatoxin concentrations are shown in Table 1. Concentrations in vegetable matter would have been greater than shown since large amounts of soil were present in the samples.

Case 3

History and Clinical Signs

Acute poisoning occurred in a group of 60 large white pigs 3 months of age which had been purchased from various

sources. The pigs were housed in dirt pens but feeding was done on concrete. A change of feed from a proprietary ration to mouldy peanut screenings was made and feed intake decreased markedly because the material was unpalatable. Three pigs were found dead 12 h after feeding and a further 5 pigs died over a period of 5 days. Many of the pigs appeared to be sick and depression, dyspnoea, apparent anaemia and dark faeces were seen. The peanut ration was replaced by the proprietary ration after 4 days and the sick pigs recovered.

Pathology

Five days after introduction of the peanut diet one sick pig was killed for autopsy. Dark yellow liver, very pale cream kidneys, pale mucus membranes and hyperaemic gastric mucosa were seen. Histological examination of the liver showed swollen eosinophilic hepatocytes with swollen vesicular nuclei and scattered bile canaliculi were dilated with yellow pigment. In the centrilobular zone there was a deficit of hepatocytes and extensive infiltration with reticuloendothelial cells (Figure 2). Many of the latter cells contained greyish yellow pigment which gave a positive reaction to Perl's Prussian Blue stain for iron. Foamy vacuolation of the cytoplasm of some centrilobular hepatocytes was also present. In the kidney the epithelial cells of proximal tubules were abnormally eosinophilic and the lumen was dilated with proteinaceous material. Obvious necrotic cell debris and neutrophils were present in scattered tubules. Mild multifocal interstitial nephritis with infiltration of reticuloendothelial cells, plasma cells and fibroblasts was present in the cortex. In the medulla protein casts were common and a diffuse interstitial infiltration with reticuloendothelial cells and fibroblasts was present. There was colonic submucosal oedema but no lesions in the small intestine examined.

Chemical Analyses

Two samples of feed with similar appearance to those of case 2 were analysed and the results are shown in Table 1.

Case 4

History and Clinical Signs

Ten large white sows, one boar and 60 growers approximately 3 months of age were housed in pens. Signs of sickness followed introduction of a new batch of sorghum into the home mixed ration. The sorghum was unripe when harvested and had a 16% moisture content soon after. Overheating had occurred in the stored grain in spite of the use of an aeration spear. Inappetance, depression and growth depression were seen in all pigs and some pigs also had diarrhoea and vomiting. Four growers died after ingesting the ration for a period of 3 weeks. Shortly after this the sorghum was mixed with an equal portion of corn but inappetance and depression continued until the sorghum was completely removed from the ration. When the pigs were slaughtered as baconers 2 carcasses were condemned due to jaundice and one of these was also cachectic.

Pathology

One of the pigs that died initially was autopsied. The liver was swollen and yellow and the kidneys were very pale. Subepicardial petechial haemorrhages and subperitoneal ecchymoses were seen. Subserosal oedema of the intestines, subepicardial oedema of the atria and perirenal oedema were present. Histological examination of the liver revealed cytoplasmic swelling and vacuolation, often with a foamy appearance, of a wide zone of centrilobular hepatocytes. Towards the periphery of the lobule hepatocytes were swollen but had less vacuolation and scattered megalocytes with basophilic cytoplasm were present. Hyperplasia of bile ductule epithelium, focal hyperplasia of Kupffer cells and scattered deposits of bile pigment in distended canaliculi were present in the periphery of the lobule (Figure 3). The epithelial cells of the proximal tubules of the kidney were swollen with vacuolated cytoplasm, lumens of numerous other tubules were dilated and sometimes contained bile and protein casts.

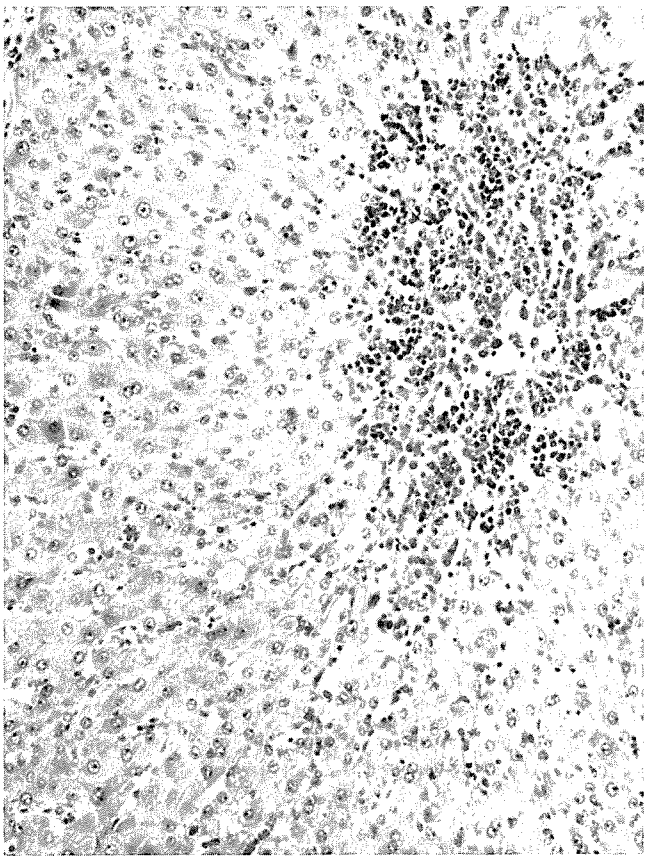

Figure 2. Hepatic centrilobular infiltration of reticuloendothelial cells — case 3. Haematoxylin and eosin X140.

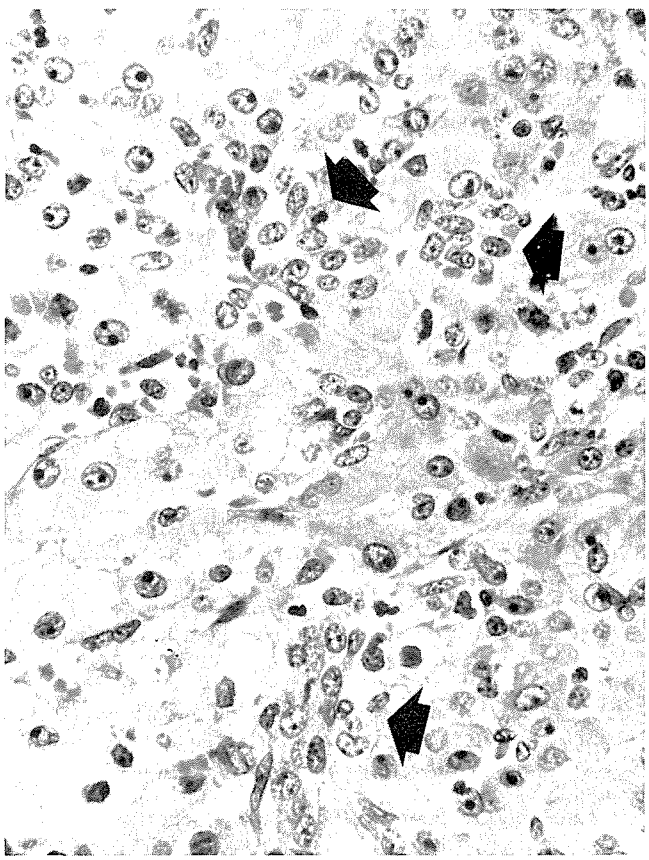

Figure 3. Bile duct hyperplasia (arrows) in the periphery of the lobule — case 4. Haematoxylin and eosin X350.

Chemical Analyses

A sample of sorghum grain used in the ration was analysed for aflatoxins and results are inlcuded in Table 1. Ochratoxin A was also detected in the sample at a concentration of 100 μg/kg.

Case 5

History and Clinical Signs

Inappetance, depression and decreased growth rate occurred in growers in a 200-sow breeding and fattening unit. This was associated with introduction of a new batch of sorghum as the grain portion of the ration. The grain was harvested before it had completely ripened and stored in a silo for less than a month before feeding. Patches of black mould growth were present in the grain. Later the grain was diluted using another batch of sorghum and fed without further problems.

Pathology

No material was available for pathological examination.

Chemical Analyses

A single sample of the sorghum grain and a pooled sample (2 kg) derived from samples collected at 13 different sites in the sorghum contained aflatoxin B_1 and B_2 (Table 1).

Discussion

Aflatoxins are products of secondary metabolism of toxigenic strains of *A. flavus* or *A. parasiticus* and can be produced in growing crops in the field or in harvested stored crops. Fungal invasion of cereal grains and peanut kernels is dependent on moisture content and temperature but seed damage due to drought, insects or mechanical trauma during harvesting also predisposes to mould growth.

Analysis of aflatoxins in feed was not done in case 1 but mouldy bread was presumed to be the source of the toxins. Bread is apparently a very suitable substrate for production of aflatoxins, possibly because of its aerated structure, and it has been the source of poisoning in several cases of aflatoxicosis in dogs (Ketterer *et al* 1975).

Peanut kernels are an excellent substrate for aflatoxin production and the peanut material fed in cases 2 and 3 were screenings from nut in shell purification procedures with a high proportion of kernel material. In addition the screenings came from a crop which was damaged by drought.

Storage problems with sorghum grain with high moisture content are a common occurrence in Queensland. The crop often is harvested late in the summer when temperatures are lower and less favourable for drying. In addition the crop tends to ripen unevenly so that frequently there is a proportion of immature grain with higher moisture content at harvest. A level of 12% moisture is considered the upper limit for safe storage (N. Heather personal communication). Aflatoxin concentration can vary greatly within a batch of mouldy sorghum as was shown by the greatly differing analysis results for sample 1, a single sample and sample 2, a pooled sample in case 5.

Relative acute toxicities in ducklings of aflatoxins B_1, B_2, G_1, and G_2 are 1.0, 0.2, 0.5 and 0.1 respectively (Carnaghan *et al* 1963). On this basis aflatoxin B_1 equivalent in the 5 stomach contents in case 1 varied from 679 to 5094 μg/kg with a mean of 2078 μg/kg. However, the peracute nature of the disease and the considerable dilution by gastric fluids suggests that much higher concentrations of aflatoxins were present in the ration fed. When the concentrations are expressed on the basis of dry organic matter in the contents, levels 10 times greater are obtained. The concentrations of aflatoxins B_1, B_2 and M_1 in livers (especially in pig no. 4) were much higher than levels previously reported for pigs poisoned with aflatoxins. When a corn based diet containing 1500 μg/kg aflatoxin B_1 was fed to 4 pigs for 35 to 42 days aflatoxin M_1 was not detected in the livers and aflatoxin B_1 was detected in only one liver at a concentration of 8 μg/kg (Stoloff and Trucksess 1979). The detection of aflatoxin B_{2a} in stored samples of stomach content is of interest. This compound is produced from aflatoxin B_1 by acid catalysed hydration and is much less toxic than the latter (Dutton and Heathcote 1966).

The average concentration of aflatoxin B_1 equivalent in the 3 samples of mouldy peanut screenings in case 2 was 22067 μg/kg and in case 3 the average in 2 samples of peanut screenings was 22675 μg/kg. Based on results of sorghum analyses and 80% sorghum level, the ration fed in case 4 contained aflatoxin B_1 equivalent of 4640 μg/kg and in case 5 the ration had 255 μg/kg (sample 2).

Pigs are among the animal species which are highly susceptible to aflatoxin poisoning. The single dose LD_{50} of aflatoxin B_1 for weaners is 620 μg/kg bodyweight and a dose of 1000 to 2000 μg/kg results in death in 18 to 24 h (Newberne and Butler 1969). It is apparent that at least the latter dose rates occurred in cases 1 and 2 where peracute disease and high mortality rates followed a single feed of toxic material. In case 3, however, where peanut material with a similar toxin level to case 2 was fed for 5 days there was a much lower mortality rate. Decreased toxin intake because of poor palatability or resistance to the effects of the toxin due to genetic factors or nutritional status may have been responsible. Protein and vitamin K levels in the diet are known to affect the toxicity of aflatoxin B_1 in pigs (Edds 1979).

Long term feeding of low levels of aflatoxins as occured in case 5 causes anorexia, decreased weight gains and decreased food conversion. Only young pigs in the herd are affected and suckers can show ill thrift which persists after weaning due to injestion of aflatoxin M_1 in the milk while sows are unaffected (Edds 1979).

Aflatoxins, notably aflatoxin B_1, primarily affect liver cells but effects on blood coagulation are also important. Peracute toxicity with extensive liver necrosis and haemorrhagic diatheses has been produced in pigs given high oral doses of crude aflatoxins or of purified aflatoxin B_1 (Wilson *et al* 1967; Cysewski *et al* 1968). Subacute toxicity with fatty infiltration and bile ductule hyperplasia in the liver and jaundice, has been produced in pigs following short term oral dosing with lower levels of crude aflatoxins (Sisk *et al* 1968). Chronic toxicity characterised by hepatic anisocytosis, fibrosis and bile ductule hyperplasia has been described following long term feeding of low levels of alfatoxins (Gagne *et al* 1968).

The differing liver pathology seen in cases 1, 3 and 4 results from the variations in toxin concentration in the feed, food intake, time of exposure and time following exposure to toxins. In case 1, where many deaths occurred within 2 h of feeding, extensive peracute liver necrosis with haemorrhage was seen. The haemorrhage seen in the intestinal lumen and the diaphragm in this peracute case was possibly caused by a direct affect on the clotting mechanism by aflatoxins. In case 3 a small proportion of pigs in the group died within 18 h of the change to toxic feed and others took up to 5 days. The pig which was examined at 5 days had a deficit of centrilobular hepatocytes with infiltration of reticuloendothelial cells containing haemosiderin indicating that centrilobular necrosis and haemorrhage had occurred. Hepatocyte swelling was present throughout the rest of the lobule and this change probably caused the bile stasis in the canaliculi. In case 4, feed with lower aflatoxin concentration was ingested for 3 weeks prior to the post mortem examination. Foamy vacuolation of a wide zone of centrilobular hepatocytes and cytoplasmic swelling of the other hepatocytes in the lobule with resulting bile stasis was seen. In the periphery of the lobule hyperplastic bile duct epithelium and scattered megalocytes were present at this stage.

Kidney tubule damage together with the typical liver lesions of aflatoxicosis have been reported with mouldy corn poisoning (Sippel *et al* 1953) and experimental aflatoxicosis (Sisk *et al* 1968) in pigs. In case 2 and case 4 of the present paper, acute kidney tubule damage was observed. The involvement

of another nephrotoxin such as the mycotoxin ochratoxin A was also considered possible and subsequently, the sorghum grain in case 4 was examined for ochratoxin A in the course of a survey of feeds for contamination by a number of mycotoxins (C. J. Moore, unpublished data). Ochratoxin A was detected at a concentration of 100 μg/kg. It has been reported by Krogh (1978) that 200 μg/kg of ochratoxin A in the ration for 4 months was required to produce kidney lesions in pigs. In case 4 it is possible that aflatoxins and ochratoxin A were additive or synergistic in their toxic effects on the kidney and there is evidence that this occurs in poultry (Huff and Doerr 1981).

The cases described have shown the potential of aflatoxin contaminated feeds to produce disease in pigs varying from a peracute fatal syndrome to a chronic ill thrift syndrome. Peanut-derived feedstuffs have long been recognised as a possible cause of aflatoxicosis but aflatoxins have also been demonstrated in a wide variety of cereals and other foodstuffs. In Queensland sorghum grain is widely used in pig rations and occurrence of 2 cases of aflatoxicosis caused by mouldy sorghum suggests that more widespread poisoning could occur if climatic and storage conditions were favourable for mould growth. Bread has very limited application as a feedstuff for pigs. However, extremely high levels of aflatoxins apparently can be produced in this material and measures should be taken to prevent this.

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