

temporary disturbance of the soft tissue (Fraser 1983). In these situations the effect of a dilution of synovial fluid on overall joint function is still to be defined.

Attempts to replace deficient or abnormal hyaluronic acid in arthritic horse joints have been described (Asheim and Lindblad 1976; Rose 1979). Suggestions that this exogenous hyaluronate is beneficial because of its visco-elastic properties or that it normalises endogenous hyaluronic acid synthesis, need further attention.

#### Acknowledgments

This work was supported by a Special Research Grant from Murdoch University. The authors wish to thank Professor J. R. Fraser of the Royal Melbourne Hospital for his advice and assistance.

#### References

- Asheim, A. and Lindblad, G. (1976) — *Acta vet. Scand.* 17: 379.  
Balazs, E. A. (1974) — *Disorders of the Knee*, Ed. J. Helfert, Philadelphia, p63.  
Balazs, E. A., Watson, D., Duff, I. F. and Roseman, S. (1967) — *Arthritis Rheum.* 10: 357.  
Bergmeyer, H. V. (Ed.) — *Methods of Analysis*, 2nd edn, New York, Academic Press Inc., p117.  
Castor, C. W., Prince, R. L. and Haxelton, M. J. (1966) — *Arthritis Rheum.* 19: 783.

- Colquhoun, D. (1971) — *Lectures on Biostatistics*, Oxford University Press, London, p160.  
Johns, R. J. and Wright, V. (1962) — *J. appl. Physiol.* 17: 824.  
McCutchen, C. W. (1959) — *Nature Lond.* 184: 1284.  
Nilsson, G. and Persson, L. (1973) — *Acta vet. Scand.* 44: 99.  
Ogston, A. G. and Stanier, J. E. (1958) — *J. Physiol. Lond.* 119: 253.  
Radin, E. L. (1972) — *Seminars in Arth. Rheum.* 2: 245.  
Radin, E. L. and Paul, I. L. (1972) — *J. Bone Jt Surg.* 54-A: 607.  
Radin, E. L., Swann, D. A. and Weisser, P. A. (1970) — *Nature Lond.* 228: 337.  
Radin, E. L., Paul, I. L., Swann, D. A. and Schotstede, E. S. (1971) — *Ann. Rheum. Dis.* 30: 322.  
Radin, E. L., Paul, I. L. and Weisser, P. A. (1971) — *Arth Rheum.* 14: 126.  
Rose, R. (1979) — *N.Z. vet. J.* 27: 5.  
Rowley, G., Antonas, K. N. and Hilbert, B. H. (1982) — *Am. J. vet. Res.* 43: 1096.  
Seppala, P. O. (1964) — *Scan. J. clin. lab. Invest.* 16 (Suppl.): 79.  
Seppala, P. O., Lehtonen, A., Karkkainen, J. and Nanto, V. (1967) — *Clin. Chim. Acta* 16: 115.  
Smith, J. W. (1956) — *J. Anat.* 90: 236.  
Sunblad, L. (1965) — *The Amino Sugars*, Ed. Balazs, E. A. and Jeanloz, R. W., New York, Academic Press.  
Swann, D. A., Radin, E. L., Nazimier, M., Weisser, P. A. Curran, N. and Lewinnek, G. (1974) — *Ann. Rheum. Dis.* 33: 318.  
Swann, D. A., Slayter, H. S. and Silver, F. H. (1981) — *J. Biol. Chem.* 256: 5921.

(Accepted for publication 17 August 1983)

## CASE REPORTS

### Zearalenone intoxication of pigs

B. J. BLANEY\*, R. C. BLOOMFIELD† and C. J. MOORE\*

**SUMMARY:** Mycotoxicosis due to ingestion of zearalenone was detected on 2 pig farms on the Atherton Tableland in northern Queensland. In one herd of 200 pigs, this resulted from feeding maize which had been stored with a high moisture content. In the other herd of 1400 pigs, it resulted from feeding sorghum grain which was rain affected before harvest. Concentrations of zearalenone in the feeds ranged up to 8 mg/kg. Most prepubertal gilts in the herds displayed enlarged teats and signs of oestrus such as having red, swollen vulvas. In several cases both rectal and vaginal prolapses occurred. On one of the farms, 25 pigs died as a direct result of prolapses. Autopsy of a 3-month-old gilt revealed apparently enlarged ovaries and uterine horns. Sows and boars seemed to be unaffected. Four gilts failed to conceive following mating during the period of zearalenone ingestion, but apart from this and the deaths from prolapses, production of the herds appeared to be unaffected.

*Aust. vet. J.* 61: 24-27

#### Introduction

The fungus *Gibberella zeae* (conidial form, *Fusarium graminearum/roseum*) is a common world-wide cause of cob rot of maize and head scab of grains such as wheat and barley. Sexual reproduction in *G. zeae* is regulated by a fungal hormone called zearalenone (Wolf and Mirocha 1977) which can be overproduced in certain circumstances. This compound can have an oestrogenic effect in some animals, affecting the reproductive system in a manner similar to oestradiol (Kurtz *et al* 1969). Pre-pubertal gilts are particularly susceptible.

In Australia the only published report of this type of effect was by Pullar and Lerew (1937), who suggested that swelling of the vulva in 2 pig herds in central Victoria was caused by

a substance present in their diet of mouldy maize. However, it was not until 25 years later that zearalenone was isolated and identified (Stob *et al* 1962). More recently, Connole *et al* (1981) reported that 2 Australian isolates of *F. equiseti* produced zearalenone in culture and we have since detected zearalenone in grain samples from central and northern Queensland during mycotoxin surveys (C. J. Moore and B. J. Blaney unpublished data).

We report here the occurrence of zearalenone intoxication in 2 pig herds on the Atherton Tableland.

#### Analytical Methods

##### *Preparation of Samples and Extraction of Zearalenone*

Feed samples were hammer milled through a 1 mm screen and then 25 g sub-samples were shaken for 30 min with 102 ml of a mixture of distilled acetonitrile: 4% aqueous potassium chloride: 5N hydrochloric acid (90:10:2). After filtration, 50 ml of filtrate was added to 50 ml of water contained in a separatory funnel and defatted by extracting it twice with 50 ml portions of distilled hexane. The hexane

\* Queensland Department of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland 4105

† Queensland Department of Primary Industries, Atherton, Queensland 4883

extracts were discarded and the acetonitrile phase was extracted twice with 50 ml portions of distilled methylene chloride. The methylene chloride extracts were passed in sequence through a 10 x 2 cm column of anhydrous sodium sulphate. The dried methylene chloride extracts were then evaporated to dryness under an air stream over a steam bath.

#### Screening Procedure for Mycotoxins

The extracts were screened for the following mycotoxins by thin-layer chromatography (TLC): aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>; ochratoxin A; trichothecene toxin T-2; sterigmatocystin; zearalenone. The extracted residue was redissolved in 0.2 ml of methylene chloride, and 10 µl of this was applied to a corner of a 10 cm x 10 cm TLC aluminium sheet, pre-coated with silica gel 60\*. The sheets were developed 2-dimensionally, first in chloroform: acetone (9:1) and then toluene: ethyl acetate: formic acid (5:4:1).

Zearalenone was visible as a blue spot when viewed under short-wavelength ultraviolet light, with an Rf of 0.90 in the first dimension, and 0.57 in the second.

#### Quantification of Zearalenone

Zearalenone was quantified by use of a high performance liquid chromatographic procedure. Suitable aliquots of the methylene chloride solution remaining from the TLC procedure were applied to a silica Sep-pak† previously flushed with chloroform, and the zearalenone was eluted with chloroform. The first 3 ml of eluate were discarded, and the next 11 ml collected, evaporated to dryness in an air stream over a steam bath and redissolved in 200 µl methanol. Aliquots (10 µl) of this solution were analysed by reversed-phase high performance liquid chromatography.

The instrument used was a Spectra-Physics 8000B fitted with F.S.970 fluorescence detector, and a Merck Hibar C-18 column operated at 35°C. The mobile phase was a mixture of methanol:water (3:1) with a flow rate of 2 ml/min. The detector was fitted with a 418 nm emission filter, with excitation at 236 nm.

### Observations and Results

#### Case 1

A herd of approximately 200 cross-bred pigs of all ages was intensively housed except for some dry sows which were run in paddocks. The diets fed were based on maize. Prior to May 1982, this maize was obtained from the Atherton Tableland Maize Marketing Board which dries, stores and markets the bulk of maize produced in the region. In April, the farmer placed 25 tonnes of farm-grown maize containing 28% moisture in a silo and attempted to dry it using only a silo aerator with a kerosene heater. One month later, another 44 tonnes of maize (17% moisture) were put in the same silo. This latter batch contained a high percentage of weed seed (*Nicandra physaloides*). At this stage a double gas burner was obtained to dry the maize.

The maize was first used in early May and several weeks later each of the growing female pigs had a swollen, red vulva while teats were also enlarged (Figure 1). These signs were not seen in suckling pigs but appeared within a week of weaning. Boars and sows seemed to be unaffected. Advice was sought when one sow aborted in August.

A sample of maize collected from the silo in August was visibly mouldy and contained about 20% of kernels with the characteristic purplish-red colour resulting from gibberella cob rot. It contained 3.3 mg/kg of zearalenone. A second 1 kg sample from the bottom of the silo contained 3.5 mg/kg while 2 samples from separate positions in the top of the silo contained 5.0 and 5.6 mg/kg of zearalenone. Samples of mixed feeds (maize plus premix) also contained the toxin:



Figure 1. Enlarged vulva and teats of a 3-month-old gilt.

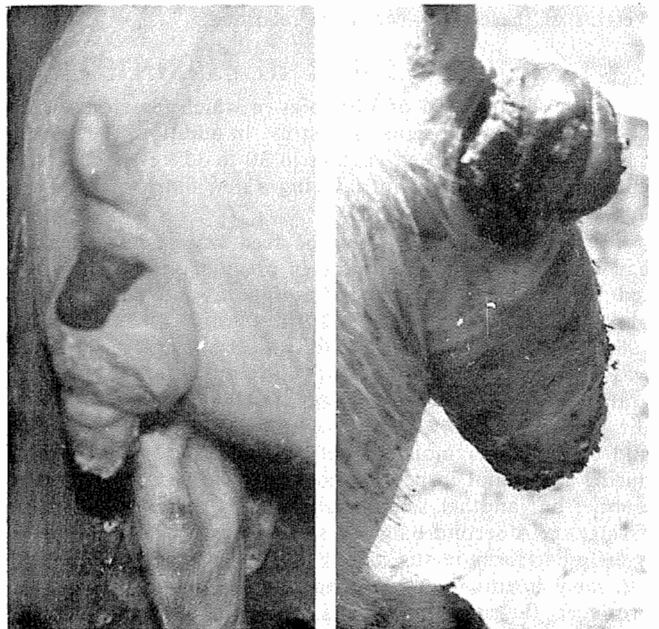


Figure 2. The early stage of vaginal prolapse and an advanced stage of both vaginal and rectal prolapse.

creep feed, 1.8 mg/kg; weaner feed, 4.5 mg/kg; grower feed, 3.2 mg/kg; sow feed, 6.4 mg/kg. No other mycotoxins were detected in any of the samples analysed.

Since no other feed was then available and because the farmer considered that the pigs' weight gains were normal, he continued to feed the mouldy maize. However, in September he reported that in 2 pens of growing pigs some had started chewing the vaginas of other pigs and that this was affecting their growth. An inspection by one of us (RCB) disclosed that this habit was induced by several cases of both vaginal and/or rectal prolapse (Figure 2). In the worst affected litter, 8 of 11 pigs died from complications arising from the prolapses; total mortality in the herd of 25 litters was 25 pigs from this cause. Two pigs with prolapses were autopsied. One pig appeared to have enlarged ovaries and uterine horns. This pig was 3 months old and had a rectal prolapse of 10 cm and a vaginal prolapse of 15 cm. In the other pig, which had a severe rectal prolapse of 20 cm plus a prolapsed vagina, the ovaries seemed slightly enlarged but the uterine horns were normal.

In October, all of the maize in the weaner feed and 55% of that in the grower feed was replaced with maize harvested in September at 15 to 16% moisture and containing 0.36

\* E. Merck, Darmstadt, Federal Republic of Germany

† Waters Associated Inc, Milford, MA01757, United States of America

mg/kg of zearalenone. The sow's diet was not changed. Two weeks after this one gilt with early vaginal prolapse had completely recovered. When inspected in December the female weaned and growing pigs had slightly swollen and red vulvas but no prolapses had occurred and they were well grown. A second sample taken from the maize harvested in September contained 0.67 mg/kg of zearalenone. The weaner feed was then replaced with another based on triticale containing no zearalenone and the signs of redness and swelling of the vulva disappeared.

Apart from the losses from prolapses production of the herd was not adversely affected. During the period in which the mouldy maize was fed, 2 sows aborted. However, the farmer reported that other sows had aborted early in the year, before the mouldy maize was fed. One sow had a pseudo-pregnancy and produced milk, but did not farrow. All other sows seemed normal. Mating and farrowings generally were normal. All sucking piglets whose dams continued to be fed the higher zearalenone diet were normal. Production records showed no effect on litter size during the period when the mouldy maize was fed. Farrowings in July, August and September included 3, 4 and 3 respectively from first litter gilts which were mated, or at least spent most of their pregnancy, while consuming the maize. However, weights of these litters were not recorded.

#### Case 2

This herd consisted of 1400 pigs in which female growing pigs were exhibiting signs of oestrus. In addition, the farmer had noticed 2 prolapsed vaginas in 30 to 35 kg Large White gilts within 3 weeks of introducing a new batch of sorghum into their diets.

The farmer suspected that the feed was responsible and had noticed a similar condition in previous years. The grain fed was a 50:50 mixture of sorghum and maize, both purchased from nearby farms. The maize was harvested in 1981, had no obvious gibberella infection and zearalenone was not detected in a sample (<0.01 mg/kg). The sorghum grain had visible mould damage and had been affected in the field by rain. It was harvested at 12 to 14% moisture and dried to 7% moisture before storage. A sample taken during the initial inspection contained 1.8 mg/kg of zearalenone, but another sample taken 3 months later contained 8.1 mg/kg. A second batch of sorghum which was grown on a different farm in the area had a similar appearance and the same history of rain damage. It was reported to have produced the same symptoms when fed previously, but a sample contained only 0.52 mg/kg of zearalenone. Samples from 2 other batches of sorghum were later submitted so that their suitability as feed could be assessed and these contained 0.62 and 0.02 mg/kg of zearalenone. These batches had not been rain affected and no mould was visible. No other mycotoxins were detected in all 4 sorghum samples.

The weight gain of growing pigs was not affected while the high zearalenone sorghum was fed and there were no infertility problems with sows or boars. However, 4 mature gilts were mated while showing signs of oestrus and did not conceive. Three of these later held to service when taken off the affected grain, while the remaining pig returned to service 3 times. One week after changing from sorghum containing 1.8-8.1 mg/kg of zearalenone to sorghum containing 0.02 mg/kg, the pigs apparently returned to normal.

#### Discussion

The typical syndrome resulting from ingestion of zearalenone was first described by McNutt *et al* (1928) who ascribed it to spoiled maize. Prepubertal gilts are worst affected with swollen vulvas, while in severe cases vaginal prolapse and on occasions rectal prolapse occurs. The immediate cause of the prolapse is probably mucosal irritation and abdominal straining, although the oestrogenic effects may cause relaxation of the rectal sphincter muscle (Kurtz and Mirocha 1978). Death results from secondary conditions such as septic cystitis,

uraemic poisoning or constipation and septicaemia following necrosis of the exposed prolapsed parts (McNutt *et al* 1928).

Daily doses of 1 mg of zearalenone which correspond to a feed concentration of about 1 mg/kg have produced swelling of the vulva in 10-week-old gilts after 8 days (Kurtz *et al* 1969). While higher concentrations produce this syndrome more quickly, the lowest concentration at which the vulva swelling can be induced has not been clearly defined. In Case 1, slightly swollen and red vulvas persisted in gilts fed a diet based on maize containing from 0.36 to 0.67 mg/kg of zearalenone, and in Case 2 the farmer had noticed signs in gilts fed sorghum when a sample of this contained only 0.52 mg/kg of zearalenone. This suggests that concentrations below 1 mg/kg may sometimes cause signs, unless the samples that we analysed were not representative of the maize consumed. There appears to be great variation in individual gilt susceptibility in field cases but this may be largely due to differences in toxin intake.

A number of other effects such as abortion, infertility, losses in production and changes in libido have been ascribed to mouldy feeds containing zearalenone. With few exceptions, reports of such effects are of little value for comparative purposes, either because the concentration of zearalenone was not determined or because the presence of other mycotoxins was not investigated. Using naturally moulded maize, Etienne and Jemmali (1982) explored the effects of zearalenone on oestrus activity and reproduction of postpubertal gilts. Their experimental diets contained 3.6 or 4.3 mg/kg of zearalenone. When fed to gilts from puberty, no swelling of the vulva occurred, but zearalenone induced a pseudo-pregnant state in 45% of the animals. No oestrus was detected within 50 days following puberty and the corpora lutea developed at puberty were maintained. The remainder, which returned to oestrus within a normal period and were then mated, had only a slightly lower conception rate than control animals. The pigs in our Case 2 either were in anovulatory oestrus and receptive to the boar, or were cycling normally but failed to conceive due to the effects of zearalenone.

Chang *et al* (1979) fed purified zearalenone at high concentrations (25, 50 and 100 mg/kg) to small groups of multiparous sows during pre-oestrus, or throughout the gestation period. These treatments produced multiple reproductive disorders including decreased litter weights, but not abortion. However, the occasional abortion reported to result from consumption of gibberella-moulded feeds containing zearalenone may be caused by various mycotoxins of the trichothecene group. One trichothecene which is commonly found in gibberella-moulded feeds in North America is deoxynivalenol (vomitoxin) which causes both emesis and feed refusal in pigs (Vesonder *et al* 1976). We did not analyse the feeds in our cases for deoxynivalenol but there was no evidence at any time of feed refusal.

*G. zeae* has been isolated in most maize growing areas in Australia (Francis and Burgess 1975). However the incidence of gibberella cob rot is increased by wet weather during maize silking, and in addition the production of zearalenone by *G. zeae* is favoured by periods of low temperature and high humidity. Zearalenone is known to be produced in maize in the field, but greatest concentrations are produced during storage at high moisture contents. In Case 1, there is little doubt that this was the major reason for the resultant zearalenone concentration, despite lack of information about the concentration at harvest. The presence of weed seed in one batch may also have inhibited the drying process by blocking aeration channels between grain kernels. Zearalenone has been detected in 28% of sorghum grain moving in commercial channels during one survey in the United States of America (Shotwell *et al* 1980), but contaminated sorghum is apparently less commonly a cause of intoxication than contaminated maize. It is not known whether zearalenone concentrations increased during storage of the sorghum in Case 2. Fungal growth should have ceased when the sorghum was dried, and so it is possible that the difference in zearalenone concentrations between samples taken 3 months

apart was due to sampling variation. The batch with the highest concentration was affected by rain before harvesting, but the precise field conditions that favour zearalenone production in sorghum remain to be clarified.

In some parts of the Atherton Tableland, maize and sorghum are grown and harvested in conditions of high rainfall and humidity and this contributed to the occurrence of cases in this locality. We believe this to be the first confirmed report of zearalenone intoxication of pigs in Australia.

#### Acknowledgments

We are grateful to P. D. Waugh for performing autopsies on 2 pigs and also to J. H. Norton, P. J. Ketterer and A. L. Tyler for their assistance.

#### References

Chang, K., Kurtz, H. J. and Mirocha, C. J. (1979) — *Am. J. vet. Res.* **40**: 1260.

- Connole, M. D., Blaney, B. J. and McEwan, T. (1981) — *Aust. vet. J.* **57**: 314.  
Etienne, M. and Jemmali, M. (1982) — *J. Anim. Sci.* **55**: 1.  
Francis, R. G. and Burgess, L. W. (1975) — *Aust. J. agric. Res.* **26**: 801.  
Kurtz, H. J. and Mirocha, C. J. (1978) — In: *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*, Vol. 2, Ed. Wyllie, T. D. and Morehouse, L. G., Marcel Dekker, NY, p256.  
Kurtz, H. J., Nairn, M. E., Nelson, G. H., Christensen, C. M. and Mirocha, C. J. (1969) — *Am. J. vet. Res.* **30**: 551.  
McNutt, S. H., Purwin, P. and Murray, C. (1928) — *J. Am. vet. med. Ass.* **73**: 484.  
Pullar, E. M. and Lerew, W. H. (1937) — *Aust. vet. J.* **13**: 28.  
Shotwell, O. L., Bennett, G. A., Goulden, M. L., Plattner, R. D. and Hesselstine, C. W. (1980) — *J. Ass. off. anal. Chem.* **63**: 922.  
Stob, M., Baldwin, R. S., Tuite, J., Andrews, F. N. and Gillette, K. G. (1962) — *Nature (London)* **196**: 1318.  
Vesonder, R. F., Ciegler, A., Jensen, A. H., Rohwedder, W. K. and Weisleder, D. (1976) — *Appl. environ. Microbiol.* **31**: 280.  
Wolf, J. C. and Mirocha, C. J. (1977) — *Appl. environ. Microbiol.* **33**: 546.

(Accepted for publication 21 July 1983)

## Chronic chondritis of the arytenoid cartilages in a pony mare

A. A. MACLEAN and R. G. ROBERTSON-SMITH

Department of Veterinary Clinical Sciences, University of Melbourne, Princes Highway, Werribee, Victoria 3030

**SUMMARY: The upper respiratory tract of a pony mare with marked exercise intolerance and respiratory stridor was examined with a flexible fiberoptoscope. Both arytenoids were adducted and distorted. A diagnosis of bilateral chondritis of the arytenoids was made and confirmed at autopsy after surgery to enlarge the rima glottidis was unsuccessful. Other space occupying lesions of the rima glottidis are discussed and theories on the aetiology are postulated.**  
*Aust. vet. J.* **61**: 27-28

#### Introduction

Space occupying lesions of the rima glottidis other than laryngeal hemiplegia are not common. Cook (1974) reported a chondroma of the left arytenoid in a 4-year-old Thoroughbred filly which was treated by unilateral arytenoidectomy. Granuloma formation following laryngotomy and laryngeal surgery (Haynes 1978) and a case of bilateral arytenoid paralysis resulting from a fracture of the cricoid cartilage with subsequent calcification (White and Blackwell 1980) have been reported. More recently Haynes *et al* (1980) reported a series of 7 cases of unilateral chondritis of the arytenoid cartilages in male Thoroughbreds. Bilateral laryngeal paralysis due to neurotoxicity from oral haloxon administration in Arabian foals has been described (Rose *et al* 1981).

This report describes the clinical features, attempted surgical repair and the pathological findings in a case of chronic chondritis of the arytenoid cartilages in a pony mare.

#### History and Clinical Examination

A 7-year-old pony mare in good bodily condition was referred with a history of progressive exercise intolerance and respiratory stridor over 3 months. The mare had suffered from an upper respiratory tract infection prior to the onset of the clinical signs. A 7-day course of corticosteroids and phenylbutazone prior to referral had produced some remission of clinical signs, but the respiratory dyspnoea recurred when the treatment was stopped.

At the time of admission, the mare was showing signs of

severe respiratory distress. The head and neck appeared normal on external clinical examination and there was no abnormal discharge from the external nares. Auscultation and percussion of the thorax revealed no abnormalities. Examination of the upper respiratory tract with a flexible fiberoptic endoscope\* revealed that the rima glottidis was reduced to a slit-like opening with apparent adduction of both arytenoids. The ability to abduct the arytenoid cartilages on inspiration and immediately after swallowing was absent. The mucous membranes of both arytenoids appeared oedematous, thickened and distorted (Figure 1). An immediate tracheostomy tube was inserted to relieve the respiratory dyspnoea.

#### Surgical Procedure

An attempt was made to increase the functional size of the rima glottidis as a salvage procedure. General anaesthesia was induced with guaiphenesin† and sodium thiopentone‡. The mare was intubated through the tracheostomy incision and anaesthesia was maintained with halothane and oxygen using a semi-closed anaesthetic system with an out-of-circle vapouriser. A left-side laryngoplasty was performed using a braided lycra prosthesis§ (Speirs 1972). There was some

\* Olympus GIF-K Olympus Optical Co Ltd, Japan.

† Gaifen, Parnell Laboratories Pty Ltd, Kirrawee, New South Wales.

‡ Intraval Sodium, May and Baker Pty Ltd, West Footscray, Victoria.

§ Dupont Lycra, Denier 2240.