Analysis of Canadian and Irish forage, oats and commercially available equine concentrate feed for pathogenic fungi and mycotoxins

Thomas Buckley, Alan Creighton, Ursula Fogarty
Irish Equine Centre, Johnstown, Naas, Co. Kildare, Ireland

Corresponding author:
Thomas Buckley, Irish Equine Centre, Johnstown, Naas, Co. Kildare, Ireland
Email: tbuckley@equine-centre.ie
Tel: +353 45 866 266
Fax: +353 45 866 701

Respiratory infections, recurrent airway obstruction (RAO) and exercise induced pulmonary haemorrhage (EIPH) are major causes of poor performance in horses. Fungi and mycotoxins are now recognised as a major cause of these conditions. The most notable fungi are *Aspergillus* and *Fusarium*. Fungal spores can originate from forage, bedding and feed and, in turn, these fungal spores can produce a series of mycotoxins as secondary metabolites.
This study set out to ascertain the degree of fungal and mycotoxin contamination in feed and fodder used in Irish racing yards over a one-year period. Weather conditions in forage producing areas were sampled by Met Eireann and the Canadian Meteorological Service.
Fifty per cent of Irish hay, 37% of haylage and 13% of Canadian hay contained pathogenic fungi. Of the mycotoxins, T2 and zearalenone were most prominent. Twenty-one per cent of Irish hay and 16% of pelleted feed contained zearalenone. Forty per cent of oats and 54% of pelleted feed contained T2 toxins.

Irish Veterinary Journal
Volume 60 Number 4, 231-236, 2007

Key words: Aspergillus, mycotoxins, RAO, EIPH

Introduction

There are two main groups of fungi for concern in the equine world. They are the field fungus *Fusarium*, which produces the toxins fumonisin, zearalenone and T2, and the storage fungus *Aspergillus*, which produces the toxins aflatoxin and ochratoxin. *Aspergillus* is a toxigenic fungus which is ubiquitous in nature. Many species are identified, yet there are only three implicated in disease: *Aspergillus fumigatus*; *Aspergillus niger*; and *Aspergillus flavus*. The other important toxigenic fungus, in relation to disease in horses, is *Fusarium* because it is a potent producer of mycotoxins.
Animals affected by mycotoxins may display symptoms such as digestive disorders, reduced feed consumption, poor thrift, impaired immunity, impaired reproduction and an undernourished appearance. They are not transmissible from animal to animal but are associated with consumption of infected feeds and forages (Quinn *et al*., 2002). Several toxins have been linked with increased incidences of cancer in humans, notably aflatoxin, fumonisin, zearalenone and ochratoxin (Jacobsen *et al*., 1999). Aflatoxin is a carcinogenic liver toxin which can suppress immunity and cause inappetance and ataxia. Fumonisins are carcinogenic to all animals and have long been associated with leucoencephalomalacia, a sporadic neurological disease in horses (Smith *et al*., 1997). Ochratoxins and T2 toxin both interfere with protein synthesis and affect immune responses by suppressing antibody formation.

A performance horse is an athlete which cannot realise its full potential if its respiratory system is not functioning to the best of its ability. During peak performance, the racing Thoroughbred will inhale and exhale up to 15 litres of air in each of 150 breaths per minute, a total of over 2,000 litres per minute (Clarke, 2001). The ability of the airways to meet those massive demands can be compromised by inflammatory and/or allergic reactions induced by infectious agents, toxins, dust, or noxious gases. Recently, there has been growing recognition that some fungi, most notably *Aspergillus* and *Fusarium* species and their mycotoxins, exert seriously deleterious effects on the performance of the equine athlete (Clarke, 2001). Recurrent airway obstruction (RAO) is an allergic response which is comparable to asthma in humans. Fungal spores, which can originate from hay, bedding and feed, are the most common cause of RAO and can also cause exercise induced pulmonary haemorrhage (EIPH), also known as bleeding (Quinn *et al.,* 2002).
Under certain conditions, fungal moulds grow unnoticed and can produce mycotoxins, toxic secondary metabolites. Mycotoxins are produced by fungi which can grow in baled hay, stored grain or silage with a high moisture content (Murphy, 1991) and it has been estimated that 25% of the world's crop production is contaminated with mycotoxins (Smith *et al*., 1994; Smith *et al*., 1997). These toxins can produce diseases known as mycotoxicosis and

IVJ_Apr_2007.indd 231 20/03/2007 12:07:28

the symptoms of such diseases vary greatly. Mycotoxin effects are species specific and produce distinctly different symptoms in various animals (Osweiler, 1996). These can include digestive disorders, reduced feed consumption, general poor thrift, an undernourished appearance, immune suppression, subnormal production, impaired reproduction and/or a mixed, infectious disease profile (Whitlow and Haglar, 2002). In livestock, the five leading mycotoxins present in feed that impair growth and disrupt reproduction are aflatoxin, zearalenone, fumonisin, T2 and ochratoxin (Diekman *et al.,* 1992). In pigs, mycotoxins impair liver and kidney function and delay blood clotting and immune responses. Even limited exposure of *Fusarium* mycotoxins to pregnant swine can result in embryonic loss and disruption of normal reproductive cycling for an extended period of time (Long *et al.*, 1983). Mycotoxins may cause similar symptoms in horses as in other animals and, currently, research into the effects of mycotoxins in this species is ongoing. At present, literature points towards mould being one of the main factors in respiratory disease in horses both by ingestion and inhalation. In time, we hope to show, through research, that ingested mycotoxins may also play a negative role in the respiratory system.

In this study, samples of hay, haylage, oats and concentrate feed were taken at intervals over a one-year period and used to estimate the possible exposure of horses to moulds and their mycotoxins. Weather data for the two harvesting periods in which the feedstuffs were produced were examined to ascertain if there was an obvious relationship with the prevalence of mould in the samples.

Materials and methods

Sampling techniques

All of the samples tested were taken over a one-year period from October 1, 2002 to September 30, 2003, from equine racing yards in Leinster and Munster with horses in training. All of the Irish forage samples had been produced in the two provinces. The Canadian forage was produced in Calgary and Lethbridge.

All forage samples were taken from the centre of bales. Approximately 100g of fodder was collected by taking 20g aliquots from five different bales, from different areas of the fodder stack. The five aliquots were pooled and placed in a sterile plastic bag which was sealed. Sterile gloves were worn at all times and they were changed between samples.

All concentrate feed samples were taken from the centre of sealed bags. Approximately 100g of feed was collected by taking 20g aliquots from five different bags. The five aliquots were pooled and placed in a sterile plastic bag which was sealed. Sterile gloves were worn at all times and changed between samples.

All of the feed/fodder samples were ground using a centrifugal ultra mill (Retsch ZM100, Germany). The screen size was 1mm and the pre-selected speed 'K' was selected at 14,000 rpm. The appropriate grinding tool was inserted depending on the type of sample. A six-tooth rotor was used for fodders and nuts and a 12-tooth rotor was used for oats and coarse mixes. When the entire sample was loaded, the apparatus was allowed to run for one minute. The sample was then removed aseptically and placed in a 150ml container. The mill was sterilised between samples using Equisept disinfectant (Thoroughbred Remedies Ireland, Co.Kildare) at a concentration of 1400 ppm. The samples were stored at between 1°C and 4°C until extraction and analysis.

Sample extraction

As several mycotoxins were being tested for, several different extraction protocols were employed (**Table 1**).

Extraction protocol 1

This protocol was used on samples being analysed for aflatoxin-HS, total aflatoxin and zearalenone. The sample material was ground as fine as possible in a food blender. Five grammes of the sample material was added to a 100ml screw cap pot. Each pot was labelled with the unique NEOGEN sample code. Twenty-five millilitres of 70% methanol were added to each pot. The pot was shaken vigorously for three minutes. The sample material was allowed to settle for two to three minutes. The extract was poured into a sterile 1.5ml eppendorf until it was almost full. Each tube was labelled with the unique NEOGEN sample code, as allocated earlier. The tube was spun in the micro-centrifuge for three minutes at 10,000rpm. The sample extract was then ready for testing.

Extraction protocol 2

Protocol 2 was identical to protocol 1 except that 25ml of distilled water, instead of the 25ml of 70% methanol, was added.

Extraction protocol 3

Protocol 3 was identical to protocol 1 except for two changes: 10g, instead of 5g, of sample was used and 40ml of 50% methanol, instead of 25ml of 70% methanol, was added.

Extraction protocol 4

Protocol 4 was identical to protocol 1, except for one change: 25ml of 50% methanol, instead of 25ml of 70% methanol, was added.

Table 1: Extraction protocols used

Sample type	Aflatoxin	Ochratoxin	Vomitoxin	Zearalenone	T2	Fumonisin
Oats	1	3	2	1	4	5
Hay/silage	1	3	2	1	4	5
Coarse mix	1	3	2	1	4	5
Cubes	1	3	2	1	4	5

IVJ_Apr_2007.indd 232 20/03/2007 15:09:05

Table 2: Forage harvest climate statistics during 2002 and a 30-year period (1961-1990) for Leinster and Munster obtained from Met Eireann and Canadian Meteorological Service

	May						June						July					
	Rainfall (mm)		Mean temp. (ºC)		Rel. humidity (%)		Rainfall (mm)		Mean temp. (ºC)		Rel.humidity (%)		Rainfall (mm)		Mean temp (ºC)		Rel.humidity (%)	
Location	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean
Birr	109.6	61.7	15.8	14.9	68.0	64.0	96.2	55.2	18.8	17.7	71.0	66.0	68.2	59.1	20.1	19.2	69.0	67.0
Dublin	121.3	55.1	14.1	14.2	72.0	67.0	81.2	56.0	17.4	17.2	73.0	68.0	68.9	49.9	19.4	18.9	71.0	68.0
Kilkenny	105.4	61.9	14.8	15.1	69.0	64.0	80.4	50.5	17.7	18.1	69.0	65.0	74.4	52.5	19.4	19.9	69.0	65.0
Mullingar	117.6	72.4	15.2	14.7	68.0	68.0	89.6	66.2	17.1	17.5	81.0	79.0	81.3	61.8	18.9	19.0	74.0	70.0
Rosslare	89.1	55.5	14.1	13.2	79.0	77.0	49.8	47.6	16.4	15.9	82.0	78.0	36.0	50.7	18.1	17.9	76.0	77.0
Cork	162.8	83.4	14.8	13.8	74.0	71.0	105.2	68.8	17.7	16.6	77.0	72.0	54.0	66.4	17.7	18.5	76.0	72.0
Shannon	139.6	60.1	15.9	15.3	68.0	64.0	89.8	62.4	18.2	17.9	71.0	67.0	68.1	57.1	18.9	19.4	71.0	68.0
MEAN	120.8	64.3	14.9	14.5	71.1	67.9	84.6	58.1	17.6	17.3	75.0	71.0	64.4	56.8	18.9	19.0	72.3	69.6
Calgary	10.9	51.4	12.9	16.4	40.6	42.8	58.6	79.8	21.3	20.2	46.0	45.8	34.6	67.9	26.1	22.9	42.4	45.7
Lethbridge	31.5	49.4	15.1	18.2	39.8	40.3	25.1	63.0	21.5	22.3	40.8	41.4	34.0	47.5	27.3	25.5	40.6	39.9
MEAN	21.2	50.4	14.0	17.3	40.2	41.7	41.9	71.3	21.4	21.3	43.4	43.6	34.3	57.7	26.7	24.2	41.5	42.8

Extraction protocol 5

Protocol 5 was identical to protocol 1 except for one change: 100 µl of the sample was extracted to a sample dilution bottle and mixed as per manufacturer's per manufacturer's (Neogen Corporation, Nellies Gate, Ayr, Scotland) instructions.

ELISA procedure

ELISA testing was carried out as per manufacturer's (Neogen Corporation, Nellies Gate, Ayr, Scotland) instructions.

Culture techniques and identification

Ten grammes of each sample was added to 90ml of sterile distilled water in a labelled 150ml container. The containers were then placed on a shaker for ten minutes. One millilitre of each of the samples was then pipetted onto a correspondingly labelled Sabouraud dextrose agar plate (Oxoid, Basingstoke). Using a spreader, the solution was spread evenly over the plates. The plates were then incubated at 25ºC for five days. After incubation, all plates were examined for fungal growth using conventional methods. The cultures were examined using the sellotape method. A loop was made with a length of sellotape. The base of the loop was touched gently onto the fungal culture so that some of the mycelium adhered to the tape. A drop of lactophenol cotton blue was placed on a microscope slide. The sellotape was placed on top of the stain and pressed down lightly. The slide was then examined under X10 and X40 magnification. The different species of fungi were characterised by colonial morphology and microscopy.

On culture, typical colonies were counted and noted. The cultures are a result of an earlier 1/10 dilution. It was decided to group the growths of fungi into light, moderate and heavy growths:

Light growth = 1-6 typical colonies = 10–60 cfu/g of sample
Medium growth = 7-12 typical colonies = 70–120 cfu/g of sample
Heavy growth = over 12 typical colonies = >120 cfu/g of sample

cfu: colony forming unit

Table 3: Cereal harvest climate statistics during 2002 and a 30-year period (1961-1990) for Leinster and Munster obtained from Met Eireann and Canadian Meteorological Service

	August						September					
	Rainfall (mm)		Mean temp (ºC)		Rel.humidity (%)		Rainfall (mm)		Mean temp (ºC)		Rel.humidity (%)	
Location	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean
Birr	44.2	77.6	18.0	18.8	68.0	68.0	22.6	70.6	16.0	16.6	71.0	72.0
Dublin	50.8	70.5	18.1	18.6	71.0	70.0	22.6	66.7	16.9	16.6	69.0	70.0
Kilkenny	52.9	69.4	19.4	19.6	64.0	66.0	19.7	73.5	17.4	17.2	68.0	69.0
Mullingar	62.9	81.2	18.6	18.6	60.0	72.0	18.9	85.9	16.7	16.4	71.0	74.0
Rosslare	40.7	68.7	17.7	17.9	75.0	78.0	12.4	73.3	16.4	16.3	76.0	76.0
Cork	47.5	88.7	18.0	18.2	73.0	73.0	35.6	96.4	16.4	16.0	72.0	76.0
Shannon	48.1	82.3	18.7	19.2	67.0	69.0	19.4	81.8	17.5	17.2	68.0	71.0
MEAN	49.6	76.9	18.4	18.7	70.0	71.0	21.6	78.3	16.8	16.6	71.0	73.0
Calgary	57.4	58.7	21.1	22.5	44.6	44.8	54.0	41.7	16.4	17.6	45.0	45.1
Lethbridge	42.4	45.1	22.0	25.4	56.5	56.8	48.3	37.6	18.8	20.1	52.9	53.1
MEAN	49.9	51.9	21.5	24.0	51.0	50.8	51.0	39.7	17.6	18.0	49.0	49.1

IVJ_Apr_2007.indd 233 20/03/2007 12:07:31

Table 4: Pathogenic fungi isolated from forage/feed during the period October 1, 2002 and September 30, 2003.

Fodder type	Number sampled	Pathogenic fungi isolated		Number *A. fumigatus*	Number *A. niger*	Number *A. flavus*	Number *Fusarium*
		No.	%				
Canadian hay	63	8	13	4	4	2	0
Irish hay	62	31	50	9	11	11	0
Haylage	54	20	37	16	2	2	0
Coarse mix	38	5	13	0	2	2	1
Oats	26	2	8	0	1	1	0
Pelleted feed	51	2	4	0	0	2	0

No. = number positive % = % positive

Meteorological data

Harvesting of forage, in the northern hemisphere, occurs predominantly in the months of May to July. Harvesting of grain crops occurs during the months of August to September. Five to seven days of good dry weather is required for haymakers. Haylage requires three to four days whereas grain harvest requires dry conditions at harvest time.

Monthly data reports of total rainfall, relative humidity and temperature for those months in 2002 were obtained from the Met Eireann and the Canadian Meteorological Service stations of the regions which the forage and crops were produced. Weather and crop reports were also obtained from Teagasc, the Irish farm advisory service, for haymaking.

Results

Tables 2 to **5** show the results obtained in the study. Note that any species of non-pathogenic fungi that were isolated (e.g., *Mucor* and most *Penicillium* sp) were not included in the results.

For each of the three months of the forage harvest period (May, June and July of 2002), data from seven weather stations in Leinster/Munster and two from Canadian areas, where the sampled forage was produced, were used to derive mean weather statistics (**Table 2**). The most striking observation is the increase in rainfall and relative humidity in 2002 compared to the 30-year mean for the Irish weather stations. During the same period in Canada, there was an overall reduction.

The results of **Table 3** show the climatic conditions from seven meteorological stations in Ireland and the two Canadian stations in August and September, detailing the rainfall, temperature and the relative humidity.

Table 4 shows the number of samples tested during the period of October 1, 2002 to September 30, 2003, including those that tested postive for pathogenic fungi. A greater percentage of Irish hay and haylage samples tested positive than the Canadian hay samples. The amount of pathogenic fungi isolated from the feed samples, i.e., oats, coarse mix and pellets, was significantly lower than in fodder. *Aspergillus fumigatus* was isolated on 16 occasions from haylage. *Fusarium*, the field fungus, was not isolated from any of the fodder samples and only once in a feed sample.

Table 5 shows the distribution of five mycotoxins in feed and forage: the number sampled, the number above the recommended level; and, the percentage above the recommended levels. The recommended levels are based on FDA (US Food and Drug Administration) and EU advisory levels for mycotoxins in horses and are as follows:

- Aflatoxin – 50 ppb (legislation)
- Zearalenone – 150 ppb (recommended)
- Fumonisin – 50 ppm (recommended by FDA)
- Ochratoxin – 20 ppb (recommended)
- T2 toxin – 150 ppb (recommended)

Aflatoxin, as seen from the data, was not an issue in the tested season. The same cannot be said for ochratoxin and fumonisin, except for the coarse mix samples which had a small number of positives. All of the feed types, apart from oats, contained positives for zearalenone with the Irish hay being the most significant. None of the forage samples contained T2 toxins but 14% of the coarse mix, 40% of the

Table 5: Mycotoxin results for forage and concentrates

Fodder type	Zearalenone		Ochratoxin		Aflatoxin		T2 Toxin		Fumonisin	
	No.	%	No.	%	No.	%	No.	P (%)	No.	%
Irish hay	44	9 (21)	15	0 (0)	44	0 (0)	15	0 (0)	15	0 (0)
Haylage	40	3 (8)	34	0 (0)	40	0 (0)	34	0 (0)	34	0 (0)
Canadian hay	65	5 (8)	0	0 (0)	65	0 (0)	0	0 (0)	0	0 (0)
Coarse mix	35	2 (6)	28	1 (4)	35	0 (0)	28	4 (14)	31	2 (6)
Oats	25	0 (0)	16	0 (0)	24	0 (0)	17	7 (40)	17	0 (0)
Pelleted feed	50	8 (16)	50	0 (0)	50	0 (0)	50	27 (54)	50	0 (0)

No. = number positive % = % positive

IVJ_Apr_2007.indd 234 20/03/2007 12:07:33

oats and 50% of pelleted feed samples tested contained T2 toxins above the advisory or recommended levels.

Discussion

Fungal spores are the most common cause of RAO (Clarke, 2001). They can also contribute to EIPH, also known as 'bleeding'. Spores are highly antigenic and cause much damage to humans and animals if inhaled or ingested. Fungi also produce mycotoxins which are toxic secondary metabolites. They are produced during the secondary sporulation stage in response to a stressful situation and so are like a defence mechanism for the fungi (Clarke, 2001). Zearalenone is an oestrogenic toxin that can lead to reproductive problems and also cause anorexia, diarrhoea and dehydration (Smith *et al*., 1997).

The fungal spores which affect horses predominantly originate from feed and fodder. It has been estimated that 25% of the world's crop production is contaminated with mycotoxins (European Commission Services, 1994). Ireland's temperate climate can often present difficulties when it comes to providing suitable feed and fodder for horses. Problems arise during the production of hay as dry weather is required to reduce moisture content to 15-20%. Haylage, however, only needs two days to wilt the grass before it is wrapped to preserve. For cereals, the weather at harvest time needs to be dry in order to reduce the moisture content to sufficiently inhibit mould growth. Toxigenic mould spores can proliferate when hay is baled damp, as can happen during a summer with a large volume of rain. If hay heats, *Aspergillus fumigatus* can often be found. Haylage may require less suitable weather but can also have problems. Producers often attempt to make hay, fail and then wrap it and call it haylage. This is unsuitable as little respiration will take place, aerobic conditions may exist and moulds will grow. Once haylage is produced, care has to be taken not to puncture the plastic that would allow both moisture and air to enter, which will encourage fungal growth. Unlike hay, once haylage is opened it has to be used within seventy-two hours or, again, fungi will proliferate.

It has been identified that wet, humid weather at flowering promotes infection by *Fusarium* of grain and grasses (Whitlow and Hagler, 2002). *Fusarium* is a field fungus and it has been shown that, whilst growing on grain, it can be inhibited and overgrown by *Aspergillus* during storage (Smith *et al*., 1997). Storage of feed and fodder is critical. High moisture and relative humidity can lead to an explosion of mould growth. Concentrated feeds are predominantly made from grains, they have their advantages but each ingredient is capable of contributing to the overall fungal and mycotoxin load. The increasing price of soya has led to some companies using citrus pulp and pine kernels instead, which may account for increased mycotoxin production. The varying stresses of heating and drying during processing of concentrates may encourage fungal damage and mycotoxin release. The competition for nutrients between fungi from harvest to storage may result in enough stresses for one or both to produce mycotoxins. There may also be a synergistic effect whereby products and toxins produced by one fungi may aid the propagation of another.

However, moisture is the undeniably important factor when discussing quality feed production for horses. Dry weather at harvest is critical in attempts to limit fungal growth and subsequent mycotoxin production. In 2002, the mean rainfall for the five weather stations examined during the fodder harvest period was well in excess of the mean for the previous thirty years for those stations. In fact, the mean rainfall in the month of May was almost double the 30-year mean for that month. During the grain harvest, the rainfall figures dropped dramatically to two-thirds of the yearly mean in August and then to a quarter of the yearly mean in September. On collating these facts with the literature, a fodder crop with high fungal activity and a grain crop with less fungal activity can be predicted. The variance between the two hays and the haylage were quite significant. Of the sixty-three Canadian hays sampled, only eight (13%) contained pathogenic fungi. Of the eight positives, 50% were *A. fumigatus* with the remaining fifty divided between *A. niger* and *A. flavus*. No *Fusarium* was isolated. The Irish hay did not fare as well. Thirty-one (50%) of the sixty-two Irish hays sampled contained pathogenic fungi. No *Fusarium* was isolated: all were *Aspergillus*, including *A. fumigatus, A. niger* and *A. flavus*. The haylage proved superior to the Irish hay but not as good as the Canadian hay. Twenty of the fifty-four sampled contained pathogenic fungi with *Aspergillus fumigatus* accounting for 80% of the positives. Again, no *Fusarium* was isolated. A number of points arise from this data. Firstly, the excessive rainfall during the fodder harvest in Ireland has contributed to the production of hay of which 50% contains pathogenic fungi. Compare this to the Canadian hay which contained 13% positives – this hay was produced in a much drier climate which has an overall 25-30% lower humidity during forage harvest periods as compared to Ireland. No *Fusarium* was isolated from forage which would suggest either none existed pre-harvest, or that *Aspergillus* propagated during storage.
The haylage showed superior results than the Irish hay at 37% positive, but it was still a very high figure. Of the positives, 80% contained *Aspergillus fumigatus* which complements the characteristic that this particular strain can survive oxygen depletion.

The results of the concentrated feed fungal analysis present a much different picture. Thirty-eight coarse mixes were sampled, of which five (13%) contained pathogenic fungi. Two contained *A. niger,* two *A. flavus* and one sample contained *Fusarium*. Twenty-six oat samples were tested with only two or 8% containing pathogenic fungi, 50% being *A. niger* and 50% being *A. flavus*. Of the fifty-one pelleted feed samples, only two, or 4%, contained pathogenic fungi - both *Aspergillus flavus*. More interestingly, the below-average rainfall seems to have contributed to a low fungal load as compared to the forage harvest. The coarse mix contained the highest percentage. The oats were next, with the pelleted feed containing the least

amount of pathogenic fungi. This would suggest that the heat treatment pelleted feed undergoes decreased the fungal load. No *Aspergillus fumigatus* was isolated but one coarse mix contained *Fusarium*. The weather at the time of harvest, coupled with good storage conditions, have contributed to concentrated feed in 2002 with a relatively low fungal load.

The literature states that zearalenone is produced by *Fusaria* spp. Only one sample contained *Fusarium* yet all the different types of feed and forage except oats contained zearalenone above the recommended level (Canadian hay 8%, Irish hay 21%, haylage 8%, coarse mix 6% and pelleted feed 16%). The Irish hay again was the worst in this regard. Five of the nine positives also contained *Aspergillus* species. This would suggest that *Fusarium* existed in both crops and forage pre-harvest, died off and produced zearalenone in doing so, with *Aspergillus* taking over as a storage fungi in some cases. Osweiller (1996) described zearalenone concentration in US hay as rarely, if ever, occurring. This is not the case in Ireland where 21% contained zearalenone above the recommended levels. None of the oats contained zearalenone, which suggests some of the other ingredients involved in production of the pelleted feed and coarse mix were responsible for the zearalenone contamination.

Fumonisin is also produced by *Fusarium* yet no samples tested contained this toxin, except in the case of 6% of the coarse mixes. Again, as the oats were negative, one of the other ingredients is responsible for the contamination. None of the feedstuffs tested positive for ochratoxin except for coarse mix, where 6% of the samples contained this toxin above the recommended level. Ochratoxin is produced by the toxigenic strains of *Aspergillu ochraceus* and *Penicillium viridicatum*, neither of which were isolated during the study. Aflatoxin is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The latter was not isolated during this study. *Aspergillus flavus*, however, was isolated on seventeen occasions throughout the study yet none of those samples contained aflatoxin above the legal/recommended levels. This would suggest that the production of the feeds and fodders did not stress the fungi sufficiently to release the toxin, nor did the fungi reach sporulation stage. T2 toxin is a trichothecene produced by *Fusarium*. It was not detected in any of the fodder samples tested. However, 14% of the coarse mix, 40% of the oats and 54% of the pelleted feeds tested contained T2 toxin above the recommended levels. This suggests that *Fusarium* was present pre-harvest and a combination of dry weather and production processes removed the fungi, but the fungi produced significant amounts of toxin which remained stable during production and storage. Another theory to add to this may be that the very moist weather early in the summer may have increased *Fusarium* growth. The dry spell which followed this may have aided the demise of the fungi resulting in a mass of toxin being produced.

Conclusions

Moulds and mycotoxins are a cause of health problems in horses and other animal species. The results of our paper show that levels of pathogenic fungi and mycotoxins are present in animal feedstuffs. Legislation is currently being drafted at EU level to address the problem of mycotoxins in animal feeds. To date, legislation only covers aflatoxins. The new legislation will cover a range of mycotoxins with acceptable levels that will need to be adhered to. Feed companies will need to have a mycotoxin screening programme on all the ingredients that make up their products. Shelf-life studies may also need to be put in place to ensure that no deterioration in feed occurs.

References

Clarke, A. (2001). Mycotoxins and their implications in the diet of performance horses. Proceedings of the 17th Equine Nutrition and Physiology Symposium. pp 1- 4.

Diekman, M.A. and Green, M.L. (1992). Mycotoxins and reproduction in domestic livestock. *Journal of Animal Science* **70**: 1615-1627.

Jacobsen, B. J., Bowen, K. L., Shelby, R. A., Deiner, U. L., and Kenuppainen, B. W. (1999). Mycotoxins and Mycotoxicoses. Alabama A&M and Aubur Universities.

Long, G.G., Diekman, M.A., Tuite, J.F., Shannon, G.M. and Vesonder, R.F. (1983). Effect of *Fusarium roseum* on pregnancy and the estrous cycle in gilts fed molded corn on day 7-17 post-oestrous. *Veterinary Research Communications* **6 (3)**: 199-204.

Murphy, D.S. (1991). Farm respiratory hazards. Fact Sheet Safety 26. Pennsylvania State University.

Osweiler, G. D. (1996). Toxicology. In: *The Five Minute Veterinary Consult.* Williams and Wilkins. pp 409-432.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. 1., and Leonard, F. C. (2002). Mycotoxins and Mycotoxicoses. *In: Veterinary Microbiology and Microbial Diseases.* Blackwell Science Ltd. pp 229–266.

Smith, J. E., Solomons, G. L., Lewis, C. W. and Anderson, J. G. (1994). Mycotoxins in Human Nutrition and Health. European Commission Study. Directorate-General XII, Science, Research and Development, Agro-Industrial Research Division.

Smith, T.K., McMillan, E.G. and Castillo, J.B. (1997). Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing DON and fusaric acid on growth and feed consumption of immature swine. *Journal of Animal Science* **75**: 2184-2191.

US Food and Drug Administration. (2001). Guidance for industry – Fumonisin levels in human foods and animal feeds. (Final Guidance). [Online]. Available from: <http://www.cfsan.fda.gov/~dms/fumongu2.html>

Whitlow, L.W. and Haglar, W.M. (2002). Mycotoxins in feeds. *Feedstuffs* **74**: 1-10.

IVJ_Apr_2007.indd 236 20/03/2007 12:07:34