# Some fungi, zearalenone and other mycotoxins in chicken rations, stock feedstuffs, lucerne and pasture grasses in the communal farming area of Rhenosterkop in South Africa

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**ABSTRACT**

Mycotoxins may be present in feeds without any visible signs of mould contamination. There is a need for rapid and accurate measurement of mycotoxins for purposes of continual monitoring and identification of high risk commodities. Samples from commercial chicken feed (maize kernels), cattle feed (lucerne, grass and hay) and milk were analysed for the presence of certain mycotoxins and cultured for fungi. Results of fungal profiles showed that most samples were contaminated by moulds belonging to the genera, *Alternaria, Aspergillus, Fusarium, Penicillium, Mucor, Phoma* and *Rhizopus.* All the chicken feed samples tested contained mycotoxins either below the recommended safe levels for poultry or below the detection limits of the ELISA tests. However, samples of grass contained levels of zearalenone greater than the allowable concentration for dairy and beef cattle (250 ppm), which may be linked to the presence of *Fusarium scirpi* identified in the fungal profiles. The levels of $AFM_1$ were below the detection limits of the ELISA tests, which may be attributed to the low levels of aflatoxins found in the feed (grass) samples. The presence of the fungus in samples analysed is not evidence for the presence of mycotoxins.

**Key words**: cattle, chickens, feed, *Fusarium scirpi,* pasture grasses, zearalenone.

Naicker D, Marias G J, van den Berg H, Masango M G **Some fungi, zearalenone and other mycotoxins in chicken rations, stock feedstuffs, lucerne and pasture grasses in the communal farming area of Rhenosterkop in South Africa**. *Journal of South African Veterinary Association* (2007) 78(2): 69–74 (En.). Division of Toxicology, Onderstepoort Veterinary Institute, Agricultural Research Council, Private Bag X5, Onderstepoort, 0110 South Africa.

## INTRODUCTION

Mycotoxins are secondary metabolites produced by filamentous fungi that have the capacity to impair animal health and productivity[6]. Fungal invasion of feed commodities and subsequent formation of mycotoxins has been identified as a prominent risk factor to feed quality with regard to feed palatability and nutritional value, ultimately having a negative effect on human and animal health[7]. Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi. Contamination may also occur during processing and storage of harvested products and feed whenever environmental conditions are ideal for fungi to develop. Moisture content and ambient temperatures are key factors of fungal colonization and mycotoxin production. These fungal toxins are chemically diverse, representing a variety of chemical families and range in molecular weight from about 200–500[26]. However, there are only a few that are considered to be of major importance based on hazard, exposure and actual levels. The more important mycotoxins, based on worldwide occurrence and intoxication, include aflatoxins, deoxynivalenol, ochratoxin A, fumonisins and zearalenone[5]. Moulds normally associated with these mycotoxins belong to the genera *Fusarium, Aspergillus* and *Penicillium*[5]. The latter 2 are associated with storage while members of *Fusarium* are usually associated with crops while still under cultivation in the field.

It is estimated by the Food and Agricultural Organization (FAO) that 25% of the world's crops are contaminated with mycotoxins[8]. In Europe and the USA, food safety drives the focus of mycotoxin research, whereas in developing countries in Africa and elsewhere, food security is of primary concern[22]. Subsistence farming is common in Africa, with home-grown crops as the only source of food, irrespective of safety and quality considerations. Rural communities are implementing small-scale maize and livestock farming to sustain their livelihoods. Their storage facilities include, amongst others, houses, tanks, plastic sheets covering the harvested crops on the ground or in sheds, and in pits, all of which can facilitate mycotoxin contamination. Aflatoxins and fumonisins are frequently associated with agricultural products in Africa. Aflatoxins are mainly present in commodities such as groundnuts, maize, sorghum and cured and smoke-dried fish[22] and fumonisins are mainly associated with maize products. Commercially produced maize seems to contain considerably lower concentrations of fumonisins compared with home-grown maize in South Africa[23].

The main aim of this study was to survey the stored feed in a rural farming community and to assess the present status of fungal amd mycotoxin contamination.

## MATERIALS AND METHODS

### *Sampling*

Six farmers identified within a 1–2 km radius in the farming community of Rhenosterkop (29.08S, 25.01E), Mpumalanga, South Africa, provide maize-based commercial chicken feed and hay, grass and lucerne for use as cattle feed. These are stored in open steel drums, in garages, under plastic sheets and in storage rooms (Table 1). Samples (top, middle and bottom) were collected from each lot and mixed to form a composite sample, containing equal portions of the entire batch. Samples were collected in sterile bags, taken to the laboratory and stored at 4 °C until further analysis. Sample bags were clearly labelled with the place and date of sampling, and the name of the farmer. Farmers were also requested to provide milk samples from their cows. These samples were collected in sterile tubes, and transported to the laboratory to screen for aflatoxin $M_1$.

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Received: April 2007. Accepted June 2007.

### *Fungal enumeration*

*Culture media*

Culture media for fungal enumeration were prepared according to the method of Rabie and co-workers[16].

*Potato dextrose agar (PDA).* The medium was prepared by adding 59 g of PDA (Difco, USA) to 1.5 ℓ distilled water and 1 capsule chloramphenicol (250 mg) (ROLAB). It was then autoclaved for 15 min and poured into 90 mm diameter Petri dishes (15–20 mℓ/Petri dish). Petri dishes were stored at 4–8 °C until used.

*Pentachloronitrobenzene agar (PCNB).* Preparation was done by dissolving Difco peptone (22.5 g), bacteriological agar (30 g), $KH_2PO_4$ (1.5 g), $MgSO_47H_2O$ (0.75 g), PCNB (3 g) and 1 capsule of chloramphenicol (250 mg, ROLAB) in 1.5 ℓ distilled water. The mixture was then autoclaved for 15 min, poured into 90 mm diameter Petri dishes and kept at room temperature to set. The Petri dishes were stored at 4–8 °C until used.

*Malt salt agar (MSA).* Malt extract (24 g) and bacteriological agar (24 g) were dissolved in 840 mℓ distilled water (A). The agar was then autoclaved for 15 min. NaCl (90 g) was dissolved in a separate container in 360 mℓ distilled water and autoclaved for 15 min (B). After cooling to 50 °C both A and B were added together. The medium was then poured into 90 mm Petri dishes and kept at room temperature to set. Petri dishes were stored at 4 to 8 °C until used.

*Sample preparation for fungal screening*

Samples that consisted of pieces of material, not milled or ground into a powder, were surface-sterilized. At least 350 pieces were placed in a sterile Erlenmeyer flask. Enough ethanol (76% v/v) was poured into the flask to cover the sample. The flask was shaken by hand for 60 s, after which the ethanol was removed. Each sample was then rinsed 3 times with sterile distilled water for approximately 1 min, placed on a clean autoclaved paper towel and covered to remove any excess water.

Milled or powdered samples were aseptically placed in sterile containers. One hundred subsamples (each the size of a knife point, from the original sample) were placed on the 3 growth media respectively.

*Assay procedure*

Twenty Petri dishes of each of the media were used per sample. Five subsamples were aseptically evenly spaced onto each Petri dish. The inoculated Petri dishes were placed in a 27 ± 3 °C incubator for at least 1 week. Petri dishes were placed under 12 h UV light-cycles to stimulate sporulation. When relatively large colonies of fungi were observed on PCNB after 1 week incubation, subcultures were made on Petri dishes containing PDA for *Fusarium* identification. Identification of mycelial fungi commenced as soon as sporulation was evident. The identification was based on morphological characteristics and how the spores were formed. The amount of kernels, pieces or subsamples infested with a specific fungal species was counted and expressed as a percentage of fungal infestation [16].

Table 1: **Sample number allocation to feed samples collected from farmers in Rhenosterkop.**

| Sample no. | Type of feed |
|---|---|
| 1 | Chicken pellets |
| 2 | Finely ground chicken feed |
| 3 | Chicken starter feed |
| 4 | Chicken grower feed |
| 5 | Cattle feed (grass) |
| 6 | Cattle feed (lucern) |
| 7 | Cattle feed – (grass)Farmer 1 |
| 8 | Cattle feed – (grass) Farmer 2 |
| 9 | Cattle feed – (grass) Farmer 1 |
| 10 | Cattle feed – (grass) Farmer 3 |
| 11 | Cattle feed – (grass) Farmer 4 |
| 12 | Cattle feed – (grass) Farmer 4 |

### *Sample preparation for mycotoxin screening*

*Dry grain samples.* The entire sample was milled with a hand mill (Sprong and Co., England) to a fine powder. The sample was mixed and 5 g or 10 g (as prescribed by the method) weighed from different locations in the milled sample. The sample was extracted as prescribed by the manufacturer (Neogen Veratox kits; Analytical Diagnostic Products, South Africa). Two chicken feed samples were collected from 1 farmer. One sample was in pellet form (sample 1) and the other was finely ground (sample 2). Samples of chicken starter (sample 3) and grower (sample 4) feed were obtained from a local supplier to the farmers, surveyed in this study.

*Grass samples.* The entire sample was milled with a Hammer mill (Raymond International, United Kingdom) to a fine powder. Preparation of samples was conducted according to the testing methods suggested by Neogen (Analytical Diagnostic Products, South Africa). Grass, hay and lucerne samples, stored in a variety of ways and intended as cattle feed, were collected from farmers and fungi present were identified.

*Milk preparation*

Farmers were requested to milk their cows. The milk was collected in sterile containers (50 mℓ) and kept refrigerated until ready for analysis. Aliquots of the samples were prepared for the detection of aflatoxin $M_1$, following the manufacturer's instructions (Ridascreen® Fast Aflatoxin $M_1$; AEC Amersham, South Africa).

*Quantification of mycotoxins using ELISA*

Using the Neogen ELISA kits, validated for maize, the chicken feed (maize-based) was analysed for some of the more common mycotoxins. The samples were screened for mycotoxins such as zearalenone, T2-toxin, ochratoxin A, total aflatoxin, deoxynivalenol (DON) and total fumonisins using direct competitive enzyme linked immunosorbent assays (Neogen Veratox kits; Analytical Diagnostic Products, South Africa). Tests were performed according to the manufacturer's instructions.

## RESULTS

### Fungal enumeration and mycotoxin detection – chicken feed

Sample 1 contained *Alternaria* spp, *Aspergillus flavus, Aspergillus terreus, Eurotium chevalieri, Mucor* spp. and *Rhizopus orysae* at high levels in the agar, which is indicative of fungal contamination (Table 2). Fungi were found in sample 2, with both PDA and MSA exhibiting 100 % of sub-samples infested with *Aspergillus flavus, Aspergillus terreus, Mucor* spp. and *Rhizopus orysa*e (Table 3). Sample 2 also exhibited 100 % infestation of MSA by *Geotrichum* spp., *Eurotium chevalieri* and *Aspergillus versicolor.*

The results for samples 3 and 4 are summarized in Table 4, and are interpreted in the same way as that of samples 1 and 2, using the fungal enumeration with the highest percentage infestation as an indicator of fungal presence. Both samples 3 and 4 contained *Fusarium verticillioides, Penicillium* spp., *Aspergillus versicolor, Eurotium repens, Aspergillus flavus, Mucor* spp. with sample 4 containing additional fungal strains of *Alternaria* and *Geotrichum.*

The ELISA results showed the presence of zearalenone, aflatoxins, deoxynivalenol and fumonisins, within allowable levels for poultry and Ochratoxin A and T2-toxin at concentrations below the limit of quantification of 2 ppb and 25 ppb, respectively (Table 5). Although samples 1 and 2 showed the highest levels of fumonisins, fungi responsible for its production were not detected.

### Fungal enumeration and mycotoxin detection – cattle feed and milk

Sample 5, representing meadow-grass

Table 2: **Percentage fungal infection of sample 1 (chicken pellets) on the 3 different media.**

| Fungal species | PDA (%) | PCNB (%) | MSA (%) |
|---|---|---|---|
| *Alternaria* spp. | 50 | – | 22 |
| *Aspergillus flavus* | 26 | – | 14 |
| *Aspergillus niger* | 16 | – | 10 |
| *Aspergillus ochraceus* | 4 | – | 2 |
| *Aspergillus terreus* | 26 | – | 72 |
| *Aspergillus versicolor* | 16 | – | 26 |
| *Cladosporium cladosporiodes* | 4 | – | 42 |
| *Eurotium chevalieri* | – | – | 80 |
| *Fusarium chlamydosporum* | 6 | – | 2 |
| *Fusarium scirpi* | 8 | – | 4 |
| *Geotrichum* spp. | – | – | 12 |
| *Mucor* spp. | 30 | – | 30 |
| *Nigrospora* spp. | 6 | – | 4 |
| *Penicillium* spp. | 18 | – | 14 |
| *Phoma sorghina* | 10 | – | 4 |
| *Rhizopus orysae* | 40 | – | 32 |

from a local supplier in Rhenosterkop, revealed mostly storage fungi. Although a wide variety of fungi was isolated from this sample, only a few were found in significant numbers. These included *Aspergillus versicolor,* as well as members of *Eurotium* and *Penicillium* (Table 4). Mycotoxins detected using the ELISA assay were all within acceptable concentrations for dairy and beef cattle (Table 5).

Sample 6, representing lucerne (from a local supplier in Rhenosterkop), contained a variety of fungi, including both storage and field fungi (Table 4). Significant levels of fungi such as *Alternaria* spp., *Fusarium scirpi, Fusarium equiseti, Penicillium* spp. and *Phoma sorghina* were isolated from this sample. Only zearalenone was found at levels of 464.9 ppb, which is greater than the safe intake level of 250 ppb for dairy and beef cattle (Table 5)[2].

A wide variety of fungi were associated with the samples 7–12. Apart from sample 7, all other samples contained high concentrations of *Alternaria.* Members of *Aspergillus, Fusarium, Mucor, Scopulariopsis, Penicillium* and *Phoma* were present in high concentrations in most of the samples. All the grass samples collected revealed levels of zearalenone greater than the recommended safe intake levels for beef and dairy cattle (250 ppb) (Table 5)[2]. Sample 10 also had levels of deoxynivalenol (DON) greater than the safe intake level of 0.3 ppm for dairy and 0.5 ppm for beef cattle[2]. Sample 11 also had high levels of aflatoxins compared with the other meadowgrass samples but within acceptable levels for consumption. According to Act 36 of 1947 ( Fertilizers, Farm Feeds, Agricultural and Stock Remedies ), the allowable level of total aflatoxin is 10–50 ppb[1]. The fungal profile of sample 11 included the presence of *Aspergillus flavus* (Table 5), which may explain the higher levels of aflatoxin detected. However, other samples that contained *Aspergillus flavus* did not have high levels of aflatoxins. In all grass samples, the total fumonisin levels were much lower than the safe intake level of 15 ppm for dairy cattle and 30 ppm for beef cattle[2]. T2-toxin levels were also much lower than the safe intake levels for dairy and beef cattle (100 ppb)[2].

Milk samples analysed showed no detectable levels of $AFM_1$ (data not shown).

Table 3: **Percentage infection of sample 2 (finely ground chicken feed) on the 3 different media.**

| Fungal species | PDA (%) | PCNB (%) | MSA (%) |
|---|---|---|---|
| *Alternaria* spp. | 60 | – | 18 |
| *Aspergillus flavus* | 100 | – | 100 |
| *Aspergillus ochraceus* | – | – | 6 |
| *Aspergillus terreus* | 100 | – | 100 |
| *Aspergillus versicolor* | – | – | 100 |
| *Eurotium chevalieri* | – | – | 100 |
| *Eurotium rubrum* | – | – | 14 |
| *Fusarium chlamydosporum* | 4 | – | – |
| *Fusarium oxysporum* | 40 | 48 | 2 |
| *Geotrichum* spp. | – | – | 100 |
| *Mucor* spp. | 100 | – | 100 |
| *Nigrospora* spp. | – | – | 2 |
| *Penicillium* spp. | – | – | 22 |
| *Rhizopus orysae* | 100 | – | 100 |
| *Trichoderma* spp. | 28 | – | – |

## DISCUSSION

At Rhenosterkop feed samples were mainly contaminated with fungi belonging to the following genera, *Alternaria, Aspergillus, Fusarium, Mucor* spp., *Phoma, Rhizopus orysae* and *Penicillium.*

*Aspergillus flavus* is known to produce aflatoxins, with a maximum acceptable level in animal feed in South Africa of 10–50 ppb[1]. In addition, *Aspergillus flavus* also produces cyclopiazonic acid[25]. Both samples 1 and 2 showed considerably high levels of *Aspergillus terreus,* which develops during storage when moisture content is above 17%. The presence of *Aspergillus terreus* is also significant as this fungus is known to produce various mycotoxins such as patulin, gliotoxin and citrinin. Both patulin and citrinin are known for their nephrotoxic effects in animals[4]. The presence of *Fusarium oxysporum* in sample 2 is important as this fungus is normally associated with diseased plant material where it can cause root rot and various other plant diseases. It can also live saprophytically on dead organic material and has been associated with the production of mycotoxins such as moniliformin and various trichothecenes. *Aspergillus versicolor* and *Eurotium chevalieri* produce the toxic metabolite sterigmatocystin, a known precursor of aflatoxins. Sample 11 contains both these fungi (Table 4), which may contribute to the high concentration of aflatoxins found in the sample (Table 5).

Extremely high levels of *Geotrichum* species were found in sample 2 (100 % in MSA) (Table 3). High levels of these fungi are known to influence the palatability of feed due to acid production[7]. Sample 2 also showed high levels of *Mucor* species (100 % in both PDA and MSA). The levels of *Rhizopus orysae* in sample 2 were found to be extremely high (100 % in PDA and MSA). Cultures of this fungus are known to be toxic to ducklings, although the mycotoxins are still unknown[17].

The very high levels of *Fusarium verticillioides* in both samples 3 and 4 are of concern as this fungus is known to infect crops in the field and propagate at moisture contents of 17% and higher. This fungus is known for the production of fumonisins, of which fumonisin $B_1$ is the most important[20]. This mycotoxin is associated with oesophageal cancer in

Table 4: **Highest percentage fungal infection of sample 3** (chicken starter feed)**, sample 4** (chicken grower feed)**, sample 5** (grass)**, sample 6** (lucerne)**, sample 7** (grass)**, sample 8** (grass)**, sample 9** (grass)**, sample 10** (grass)**, sample 11** (grass)**, sample 12** (grass) **on the 3 different media.**

| Fungus | Sample 3 Chicken starter feed | Sample 4 Chicken grower feed | Sample 5 Grass | Sample 6 Lucerne | Sample 7 Grass | Sample 8 Grass | Sample 9 Grass | Sample 10 Grass | Sample 1 Grass | Sample 12 Grass |
|---|---|---|---|---|---|---|---|---|---|---|
| *Alternaria* spp. | – | 52% MSA | – | 22% PDA<br>54% MSA | – | 100% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA | 00% PDA<br>MSA | 100% PDA<br>MSA |
| *Aspergillus carbonarius* | – | – | – | – | 76% PDA<br>72% MSA | – | – | – | 100% PDA | – |
| *Aspergillus flavus* | 62% PDA<br>70% MSA | 92% PDA<br>86% MSA | – | – | 62% PDA<br>58% MSA | – | – | – | 40% PDA<br>38% MSA | 36% PDA<br>40% MSA |
| *Aspergillus nygamai* | – | – | – | – | 100% PDA<br>MSA<br>PCNB | – | 100% PDA<br>90% PCNB | 100% PDA<br>MSA<br>PCNB | – | – |
| *Aspergillus ochraceus* | – | – | – | – | 80% PDA<br>76% MSA | 66% PDA<br>60% MSA | 100% PDA<br>MSA | – | 100% PDA<br>MSA | 100% PDA<br>MSA |
| *Aspergillus versicolor* | 70% MSA | 82% MSA | 44% MSA | – | – | 54% MSA | 58% MSA | 100% MSA | – | – |
| *Cladosporium cladosporiodes* | – | – | – | 100% MSA | – | – | – | – | – | – |
| *Eurotium amstelodami* | – | – | 46% MSA | – | – | – | – | – | – | – |
| *Eurotium chevalieri* | – | – | – | – | – | 54% MSA | 100% MSA | – | 100% MSA | 100% MSA |
| *Eurotium repens* | 60% MSA | 76% MSA | – | – | – | – | – | – | – | – |
| *Fusarium chlamydosporum* | – | – | – | – | – | 86% PDA<br>84% MSA<br>90% PCNB | – | – | – | – |
| *Fusarium equiseti* | – | – | – | 44% PDA<br>44% MSA<br>90% PCNB | – | – | – | – | – | 92% PDA<br>100% MSA<br>100% PCNB |
| *Fusarium scirpi* | – | – | – | 36% MSA<br>46% PCNB | – | 100% PDA<br>MSA<br>PCNB | 90% PDA<br>94% MSA<br>88% PCNB | 80% PDA<br>MSA | 100% PDA<br>78% MSA<br>PCNB | 40% MSA<br>40% PCNB |
| *Fusarium verticillioides* | 100% MSA<br>PDA<br>PCNB | 100% MSA<br>PDA<br>PCNB | – | – | – | – | – | – | – | – |
| *Geotrichum* | | 100% MSA | – | – | – | – | – | – | – | – |
| *Mucor* spp. | 100% PDA<br>MSA | 100% PDA<br>MSA | – | – | 100% PDA<br>MSA | 80% PDA<br>76% MSA | 100% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA |
| *Penicillium* spp. | 100% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA | 44% PDA<br>93% MSA | 80% PDA<br>74% MSA | 100% PDA<br>MSA | 50% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA | 100% PDA<br>MSA |
| *Phoma sorghina* | – | – | – | 100% PDA | – | 100% PDA | 68% PDA | 100% PDA | 100% PDA<br>MSA | 86% PDA |
| *Rhizopus orysae* | – | – | – | – | 100% PDA | 80% PDA | 100% PDA | – | – | 100% PDA<br>MSA |
| *Scopulariopsis* spp. | 88% MSA | – | – | – | 100% PCNB | 100% PCNB | 100% PCNB | 100% PCNB | 100% PCNB | 100% PCNB |
| *Trichoderma reesei* | – | – | – | – | | 60% PDA | – | – | 60% PCNB | – |

Table 5: **Mycotoxin levels detected in all samples using ELISA.**

| Sample | Zea (ppb) | Afla (ppb) | Ochra A (ppb) | T2-Toxin (ppb) | DON (ppm) | Fumonisins (ppm) |
|---|---|---|---|---|---|---|
| 1. Chicken pellets | 319.1 | 2.6 | Nd | Nd | 0.6 | 8.7 |
| 2. Chicken feed | 165.4 | 2.9 | Nd | Nd | 0.8 | 8.3 |
| 3. Chicken starter feed | 146.4 | 3.3 | Nd | Nd | 0.5 | 6.8 |
| 4. Chicken grower feed | 130.1 | 3.0 | Nd | Nd | 0.7 | 6.2 |
| 5. Cattle feed (grass) | 234.5 | 4.6 | Nd | Nd | 0.4 | 1.9 |
| 6. Cattle feed (lucerne) | 464.9* | 2.3 | Nd | Nd | Nd | 1.2 |
| 7. (grass) | 445.8* | 14.1 | 5.2 | 34.6 | 0.3 | 2.1 |
| 8. (grass) | 1037.4* | 19.0 | Nd | Nd | Nd | Nd |
| 9. (grass) | 488.4* | 9.9 | 5.9 | Nd | Nd | Nd |
| 10. (grass) | 1198* | 10.7 | Nd | Nd | 1.5* | 7.6 |
| 11. (grass) | 444.2* | 21.4 | Nd | Nd | Nd | Nd |
| 12. (grass) | 407.2* | 10.9 | 4.6 | 42.7 | 0.4 | Nd |

*Above the safe intake level[2].
Nd – not detectable.

humans and can cause leucoencephalomalacia in horses, mules and donkeys, and is toxic to ducklings[13,24]. The very high levels of *Geotrichum* spp. in sample 4 (Table 4) indicates that feed refusal could be experienced when fed to animals due to acid production. Both samples 3 and 4 showed a high infestation by members of *Mucor* (Table 4). Both samples also contained very high levels of *Penicillium* species, which are known to produce a wide variety of mycotoxins that can range from almost non-toxic to highly toxic[21]. *Scopulariopsis* spp. was found in high levels in sample 3, a further indication that the sample was exposed to high moisture contents above 17 % for a considerable time.

*Alternaria* species normally develop under conditions of high moisture in the field and are known to produce mycotoxins, alternariol, alternariol methyl ether and tenuazonic acid[10]. The presence of the fungus can cause the commodity to have a darkish colour. The presence of *Phoma sorghina* in some samples also indicates that tenuazonic acid, which is responsible for the inhibition of protein synthesis, could be present[18]. In addition, this fungus can also produce an unknown mycotoxin affecting blood platelet counts in humans and animals, leading to thrombocytopenia and subsequent haemorrhaging[19].

The levels of aflatoxins found are of not much concern; however, aflatoxin-contaminated feed does not only reduce animal performance and overall health, but also creates risks of residues in milk[7]. In beef cattle, calves are more sensitive to feed contamination than adult cattle. Feeding high levels of aflatoxins can cause liver damage and depress the immune system, resulting in disease outbreaks[9].

Aflatoxin $M_1$ is produced in the liver of animals and humans that have ingested aflatoxin contaminated commodities, as a hydroxylated metabolite of aflatoxin $B_1$[12]. It is normally excreted in the urine and the milk of dairy cattle and other lactating mammals. The occurrence of $AFM_1$ in milk is transitory in nature, usually reaching a peak within 2 days of ingestion of the contaminated commodity and disappearing within 4 to 5 days after the withdrawal of the contaminated source[11]. Several authors have examined the relationship between the dietary intake of aflatoxin and the excretion of $AFM_1$ into milk[3,15]. Their data suggest that there is a linear relationship between the total dietary intake of aflatoxin $B_1$ and the concentration of $AFM_1$ in the milk(which milk). It has been suggested that it takes 4–6 days of regular consumption of aflatoxin before steady state excretion of aflatoxins in milk is achieved[14]. It has been concluded that when the daily intake of aflatoxin $B_1$ is below 0.07 mg, virtually no $AFM_1$ can be detected in the milk[11]. The FDA regulation in milk is 0.5 ppb [12]. The grass samples, collected and analysed for fungi and mycotoxins, showed that although low concentrations of aflatoxin $B_1$ were detected, they did not result in detectable levels of $AFM_1$ in the milk.

The presence of *Fusarium scirpi* in significant numbers (Table 4) poses a risk that diacetoxyscirpenol (DAS) could be formed in samples. This mycotoxin is a member of the trichothecenes, which are responsible for haemorrhaging and dermal toxicity in humans and animals.

The presence of *Fusarium scirpi* in significant numbers in samples 6, 8, 9,10,11 and 12 (Table 4) may explain the high concentrations of zearalenone found (Table 5). The unexpectedly high levels of zearalenone recorded in the samples of pasture grass (samples 8, 9, 10 and 11) and lucerne (sample 6) may be attributable to colonisation by *Fusarium scirpi.* According to a recent review, zearalenone has been reported only in relation to porcine hyperoestrogenism in South Africa[12]. The authors quote New Zealand data that contamination of pastures by zearalenone adversely effects fertility in ewes. In New Zealand, high levels of zearalenone were demonstrated in samples of *Fusarium*-infected pastures associated with infertility, while $\alpha$- and $\beta$-zearalenone (metabolites of zearalenone) could be detected in the urine of affected sheep. The high zearalenone levels coincided with the sheep breeding season when exposure to the toxin would be the highest. Dosing trials subsequently revealed that about 3 mg zearalenone per sheep per day before mating depressed the ovulation rate and lowered lambing percentages[12]. Some of the other responses in ruminants to zearalenone poisoning reportedly include reduced feed intake, poor reproductive performance and mammary enlargement in virgin heifers[9]. Since low fertility is generally regarded as being the main limiting factor for livestock production in South Africa, the significance of toxic levels of zearalenone in South African pastures and the scope of the contamination must be urgently investigated.

Although the presence of the fungus is not evidence for the presence of the relevant mycotoxins, a real risk exists that mycotoxin formation can take place under ideal environmental conditions. Analysing only for the known mycotoxins could lead to a misperception that a commodity is safe for human or animal consumption although many unknown and undetectable secondary fungal metabolites could be present.

## ACKNOWLEDGEMENTS

We wish to thank the Gauteng Provincial Government for the financial assistance to carry out this study, and we also wish to acknowledge the rural farming community of Rhenosterkop for their willingness to participate in the study.

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