

# ACTS AND REGULATIONS

## CODE OF PRACTICE FOR THE CONTROL OF MYCOTOXINS IN THE PRODUCTION OF ANIMAL FEED FOR LIVESTOCK

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### PREFACE

The purpose of AFMA's Code of Practice for the Control of Mycotoxins in the Production of Animal Feed for Livestock is to:

- Provide an Overview on Mycotoxins;
- Provide guidelines for establishing Good Practices for the Control of Mycotoxins in the Feed Industry; and
- Provide interim guidelines on Maximum Acceptable Levels of Mycotoxins in Animal Feeds until Local and/or Internationally Accepted Regulations are set.

This Code will be reviewed on an annual basis by the AFMA Technical Committee and will be based on South African Regulations and will also take guidance from the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations as well as from the United States Food & Drug Administration.

### GENERAL

**Mycotoxin:** Naturally occurring toxic metabolite of certain moulds that are not intentionally added to feeds or feedstuffs.

**Maximum Acceptable Level (MAL):** Maximum concentration of the specific mycotoxin in a feed recommended by AFMA to be acceptable, based on levels acceptable worldwide.

### OVERVIEW OF MYCOTOXINS

The term mycotoxin is derived from "myco", from the division Eumycota to which moulds belong, and "toxin", meaning poison. They are produced by moulds that grow on crops before or after harvest and on feeds and other organic materials during storage. There are over 70 000 different mould species. Moulds or mould spores are common in commodities and feeds and mould growth lowers the nutritive value of the products on which they grow and can result in feed refusal due to a change in colour, consistency and smell. The Food and Agriculture Organization (FAO) of the United Nations has estimated that 25% of the world's food crops are contaminated by mycotoxins each year.

The mycotoxins are secondary metabolites that affect both human and animal health and can lead to great economic losses in animal production. The three main genera of mycotoxin producing moulds are *Aspergillus*, *Fusarium* and *Penicillium*. Mycotoxins may be carcinogenic (aflatoxin B1, ochratoxin A, fumonisin B1), oestrogenic (zearalenone), neurotoxic (fumonisin B1), nephrotoxic (ochratoxins, citrinin, oosporeine), dermonecrotic (trichothecenes) or immunosuppressive (aflatoxin B1, ochratoxin A, T-2 Toxin). Most are relatively stable and are not destroyed by processing.

Mycotoxins are a threat both in terms of their impact on animal production and the threat to human health. Mycotoxins can be passed through the food chain to humans via residues in carcasses, organs, eggs and milk. Fortunately healthy animals tend to detoxify most of the mycotoxins to which they are exposed, although some of the end-products of this process are also toxic (e.g. Aflatoxins M1 & M2 in milk). The resulting mycotoxin residues in food therefore tend to be considerably lower than the levels in the feed consumed by the animals, thus acute intoxication in humans from these residues is unlikely.

There is a significant economic impact of mycotoxins in animal production through disease and impaired performance. The incidence of mycotoxin production by moulds varies among commodities, climatic conditions and regions with optimum conditions for mycotoxin production being different for each mould. As the environment plays such an important role in the occurrence of mycotoxins the incidence of mycotoxin contamination of a specific commodity can vary considerably from year to year and from region to region. Their effect on animal production is influenced by concentration of toxin, exposure period, presence of other toxins, age of animal, and the nutritional and health status of animal. The effect is more often chronic rather than acute, with immune suppression being the typical symptom together with damage to vital organs and reproduction capacity. Mycotoxins tend to be low molecular weight compounds which are not detected by the body's antigens. Thus, unlike bacterial toxins that are proteins, there is no antibody mediated response to mycotoxins with typical symptoms. Mycotoxins tend to be more insidious poisons. These factors make determination of the actual economic impact difficult.

There are over 500 known mycotoxins with the most important based on toxicity and prevalence being aflatoxin, deoxynivalenol (DON or vomitoxin), zearalenone, fumonisin, ochratoxin, T-2 toxin and T-2-like toxins (trichothecenes). Moulds of the genus *Fusarium* produce over 70 different mycotoxins, with some strains capable of producing up to 17 mycotoxins simultaneously. Although some species of animals are less susceptible to certain mycotoxins than others, the presence of mould should be a concern due to reduced nutritive value, palatability and possible presence of numerous other mycotoxins. There are also so-called masked mycotoxins that are bound and only released during digestion and thus go undetected by routine screening techniques.

Sensitivity to the various mycotoxins differs between the animal species. Horses are more susceptible to mycotoxins than ruminants since nutrient absorption occurs prior to the fermentative digestion process of the large intestine. Ruminants are generally the least sensitive to mycotoxins. Swine tend to be more sensitive than poultry, especially nursing or nursery aged swine. Differences within poultry exist with ducklings reported to be 5-15 times more sensitive to aflatoxin than are laying hens and some laying strains 3 times more sensitive than others. Poultry species tend to be less sensitive than other species to fumonisin, DON and zearalenone.

With over 500 mycotoxins having been identified the presence of a mycotoxin in a sample is only an indication of contamination as there is a strong likelihood of the presence of other, possibly synergistic, mycotoxins. This is particularly true in mixed feeds where various feed components contribute to the mixture of mycotoxins. This, together with the typical uneven distribution of mycotoxins, requires that maximum acceptable levels be set conservatively in the industry. The control of the problem will however require the commitment from all involved from crop grower through to animal production manager.

It would appear from the statistics on mycotoxin contamination in mixed feeds and major feed ingredients (Maize and Soybean meal) that the focus of attention should be on the moulds that produce deoxynivalenol, fumonisin and zearalenone and on the conditions under which these mycotoxins are produced. These are toxins produced by *fusarium* spp. which are plant pathogens, the toxin contamination thus occurring pre-harvest.

We need to clearly establish the threat and find practical solutions. However, we must also be wary of fuelling a crisis by raising the issue of mycotoxins unnecessarily. We must also be mindful of the fact that the increased costs resulting from prevention or control of mycotoxin occurrence will ultimately be passed on to the consumer. Such control requires accurate assessment through sophisticated sampling, sample preparation, extraction and analytical techniques. Because mycotoxins cannot be totally eliminated the maximum acceptable levels must have a reasonable expectation of being achieved and being reliably measured. The technological possibilities to comply with the maximum acceptable levels must be taken into account.

## **CONTROL**

Given the nature of the problem we must accept that prevention is better than cure. It is thus imperative that all involved from crop grower through storage handler and feed mill operator to the livestock producer accept responsibility. Moulds, and in particular mould spores, are unavoidable contaminants in feedstuffs. The approach must be to reduce the possibility of contamination at each critical point in the chain. The principles used to reduce mycotoxin contamination must be:

- preventing contamination at source;
- applying appropriate technology in crop production, handling, storage and processing;
- applying measures aimed at decontamination and measures to prevent marketing of contaminated feed and feedstuffs.

### **Pre-Harvest Control**

The primary objective is to reduce mould growth and thereby reduce the presence of mycotoxins. Moulds are part of the natural ecology of the soil, thus a reduction or prevention program must start here. Land preparation is the first step in the process. The cost-saving method of minimum or zero tillage leaves organic matter at or near the soil surface, thereby increasing the likelihood of the growth of systemic moulds such as *Diplodia* and *fusarium* spp. the use of mould resistant varieties and treated seeds can reduce the incidence of mould colonization. Adequate soil nutrient supplementation will reduce crop stress and the incidence of seed splitting, thereby reducing the risk of mould colonization of the plants. Crop rotation reduces the carry-over of pathogenic moulds between successive crops. High acidic, compacted soils cause the loss of competitive exclusion and also increase the risk of colonization by pathogenic moulds such as *fusarium* spp. Chemical protection of crops against insect damage (which facilitates mould colonization) and moulds are available to control mycotoxin contamination at source for the most prevalent mycotoxins in South African feedstuffs and which are produced predominantly by *fusarium* spp.

### **Harvest Control**

Harvesting of crops under the correct conditions and at the correct moisture will reduce the risk of mould colonization. Harvest equipment should be clean of old organic matter so as to minimize the risk of cross-contamination from previous crops. Machinery should also be correctly set so as to minimize grain damage and thus the risk of mould colonization.

### **Storage Control**

The most critical control point in the prevention of post-harvest mould growth and mycotoxin production is at the receipt of materials at storage facilities. This applies to grain storage silos as well as feed mill storage facilities (both on- and off-site). All handling and storage facilities must be clean prior to the intake of material. A "dead" spot in a conveyor system where organic matter accumulates and becomes colonized by a toxigenic mould will distribute spores over all fresh material passing that point. Such areas should be identified in a HACCP program. Regular cleaning and treatment with a mould inhibitor (e.g. in dry powder form) will reduce or prevent mould colonization. Material with obvious mould contamination should be rejected and not simply downgraded. Such material, if taken into storage, will serve as a source of contamination of clean material and negate cleaning and hygiene efforts.

An important and effective method of minimizing mould contamination in grains is to clean out broken kernels which are prone to colonization and to separate out infected grains so as to prevent cross-contamination. This can be achieved by means of sieves.

All commodities being received into storage must be dried to levels recommended for the specific commodity. Generally, whole grains should be stored at a moisture level of below 14%, although levels of 11% and 9% have been quoted for soybeans and groundnuts respectively. The moisture level for other feedstuffs such as grain by-products, proteinacious ingredients and animal feeds to be stored should not exceed 12%. A mould inhibitor should be included for ingredients that have to be taken in or feeds dispatched with moisture levels exceeding the above mentioned guidelines. Moisture levels of 12-15% are favourable for insect development, with little insect activity when

grain moisture levels are below 9%. Reducing the risk of insect damage will also reduce the risk of mould colonization and mycotoxin production.

Temperature control of stored commodities is another critical aspect in the control of mould and mycotoxins. The ideal temperature range for mould propagation is 25-30 °C with 18 °C being the recommended maximum for stored commodities. Temperature control can be achieved with forced air systems and/or stock rotation within the storage facility. Commodities stored in bags should be stacked on pallets and stand free of walls and ceilings and be protected from moisture damage.

### **Feed Manufacturing Control**

The control procedures discussed under storage are applicable to feed manufacturing facilities and feed delivery vehicles. This includes control of moisture of material to be stored, aeration of storage silo's to reduce moisture migration and keep the material dry, avoiding broken kernels (they have 5 x greater risk of mould growth) and cooling pelleted feed adequately before storage (particularly in metal bins). Particular attention should be given to the pelleting process to ensure that the 3-4% moisture added to feed during conditioning prior to pelleting is removed in the cooling process and that the temperature of the pellets is reduced to no more than 6 °C above ambient. Excess moisture together with higher pellet temperature will result in condensation in conveying and storage facilities, thereby promoting mould colonization.

High-risk ingredients should be monitored for moisture and mycotoxins. High-risk materials are identified on the basis of the prevalence of contamination, inclusion levels in feeds and species being fed. Cereals are thus high risk due to their high inclusion rates even if the prevalence and levels of mycotoxins is low. Strict first-in-first-out inventory procedures with minimal storage periods must be followed.

At the feed mill tests can be conducted for the presence of a handful of the major mycotoxins. Although the HPLC remains the AOAC approved method of testing there are quicker and less expensive test kits available for some of the more important mycotoxins. Sampling technique is critical as the mycotoxins are not evenly distributed in the material.

### **Livestock Production Facilities Control**

The feed storage and conveying systems have exactly the same requirements as those for storage in terms of the control and prevention of mould colonization and mycotoxin production. Under favourable conditions of moisture and temperature mould colonization and mycotoxin production can develop within 48 hours. Feed in bins exposed to sunlight is prone to moisture migration. The feed thus tends to cake on the bin walls, particularly in corrugated bins. This feed becomes mouldy and then continually seeds fresh feed with spores. Humidity and temperature levels in confined housing systems are relatively high. Feed in such an environment will gain moisture and be prone to mould colonization and mycotoxin contamination. Feed storage systems must thus operate on a first-in-first-out system, with fresh feed being delivered every 10 days. These aspects can be facilitated by appropriate ordering procedures and twin-bin installations to allow for rotation and periodic bin cleaning.

### **GUIDELINE MAXIMUM ACCEPTABLE LEVELS (MAL's)**

Determination of maximum acceptable levels for mycotoxins is not an easy task and "no observable effects levels" (NOEL) of the various mycotoxins do not exist for each animal species. There is also no single internationally accepted regulation on maximum levels in commodities and animal feeds. There is a wealth of information on aflatoxins since they were the first to be discovered in 1961. The maximum levels for these mycotoxins have been set by a number of regulatory bodies. At present the regulations controlling animal feeds in South Africa only sets limits for these mycotoxins. Information on tolerance levels of the more recently discovered mycotoxins (e.g. fumonisin in 1988) is scarce.

A further complication is that the effects of mycotoxins are influenced by hygiene status, health status and, in particular, stocking density. Determination of tolerance levels under controlled conditions on purified mycotoxins gives tolerance levels far higher than those observed in field conditions. There is also evidence of synergistic effects of the mycotoxins, with the presence of fusaric acid reported to increase the toxicity of trichothecenes. It is also unlikely that individual mycotoxins occur in isolation in field conditions. Isolation of a mycotoxin in a sample is only an indication of mycotoxin contamination as there may be other unknown mycotoxins and even masked mycotoxins present. Determination of tolerances from field trials thus also produce diverse results.

Mycotoxins cause a variety of adverse symptoms in animal production, but acute toxicosis and death are infrequent. The symptoms are normally non-specific effects associated with reduced performance and increased disease susceptibility. The immune system is the first to suffer through depressed lymphocyte activity, suppressed antibody production and impaired macrophage function. The answer on maximum acceptable levels must therefore be as low as possible. The following information on the most important mycotoxins can only serve as a guideline. The levels may be seen as conservative so as to allow for non-uniform distribution in feed, the likelihood of the simultaneous presence of other mycotoxins and the risks to human health. These levels are unlikely to result in impaired animal performance. The following principles proposed by Codex Alimentarius Commission of the FAO have been considered:

- MAL's shall only be set for those contaminants that present a significant risk;
- MAL's shall be set as low as reasonably achievable. MAL's shall be set at a level which is slightly higher than the normal range of variation in levels in foods that are produced with current adequate technological methods, in order to avoid undue disruptions of food production and trade (providing these levels are acceptable from the toxicological point of view);
- Proposals for MAL's in products shall be based on data from at least various countries and sources, encompassing the main production areas/processes of those products;
- MAL's shall apply to representative samples per lot;
- MAL's should not be lower than a level which can be analyzed with methods of analysis that can be readily applied in normal product control laboratories, unless public health considerations necessitate a lower detection limit which can only be controlled by means of a more elaborate method of analysis.

<b>Species</b>	<b>Aflatoxins (7,10)</b>	<b>Fumonisin (4,7,10,11)</b>	<b>DON (4,7,10)</b>	<b>Zearalenone (4,7,8)</b>	<b>T-2 toxin (4,7)</b>	<b>Ochratoxins (4)</b>	<b>DAS (4)</b>	<b>Citrinin (4)</b>
<b>Poultry</b>								
Broilers	20 ppb	50 ppm	2 ppm	>800 ppm	0.4 ppm	0.5 ppm	0.4 ppm	>250 ppm
Layers	20 ppb	15 ppm	5 ppm		1 ppm	0.5 ppm	0.5 ppm	
Ducks	20 ppb	50 ppm	2 ppm					
<b>Swine:</b>								
Nursing	20 ppb	10 ppm	0.3 ppm	0.2 ppm	0.2 ppm	0.2 ppm		
Growing	20 ppb	10 ppm	0.3 ppm	0.2 ppm	0.2 ppm			
Sow	20 ppb	10 ppm	0.3 ppm	0.1 ppm	0.2 ppm			
<b>Cattle:</b>								
Dairy	20 ppb	15 ppm	0.3 ppm	0.25 ppm	0.1 ppm			
Beef	20 ppb	30 ppm	0.5 ppm	0.25 ppm	0.1 ppm			
Sheep	20 ppb	30 ppm	0.5 ppm	0.25 ppm				
Horse	20 ppb	1 ppm	1 ppm	0.1 ppm				
Rabbit	20 ppb	1 ppm	1 ppm	0.5 ppm				
Catfish	20 ppb	10 ppm	1 ppm	0.5 ppm				
Dogs	20 ppb	5 ppm	1 ppm	0.5 ppm				
Cats	20 ppb	5 ppm	1 ppm	0.5 ppm				

The recommendation for Ergot is a maximum of 1000 ppm in all feeds. No recommendations are available for the prevention of Diplodiosis as the causative mycotoxin has not yet been identified.

## **SAMPLING & TESTING**

Mycotoxins may be present in feedstuffs without any visible sign of mould contamination. There is thus a need for rapid and accurate measurement of mycotoxins for purposes of continual monitoring and identification of risk commodities. It is essential that internationally approved analytical methods be employed. Quick tests can be used to identify potential risks, but where significant levels of mycotoxin are found the levels (in the same sample) should be verified using methods accepted by the Association of Official Analytical Chemists (AOAC).

Sampling is the most critical step in determining the presence and levels of mycotoxin contamination since the distribution of mycotoxins is generally uneven in commodities or feedstuffs. Results on samples that are not representative of the bulk are meaningless. In order to obtain a sample representative of a lot sufficient number of samples must be taken, carefully mixed and then a laboratory sample ( $\pm$  2kg) obtained by successive divisions by means of mechanical dividers or a "quartering" technique. Sampling of bulk material must be by means of cylindrical probe samplers. Samples should be taken at points 2m apart and to the depth of the container where practical. Samples from bagged material must be obtained by means of a sack-type spear sampler. The number of bags to be sampled is given in the table below.

<b>Total No. of Bags</b>	<b>No. of Bags to be Sampled</b>
Up to 10	Every Bag
10 to 100	10 - Randomly selected
More than 100	$\pm$ Square root of total number - Randomly selected across consignment or lot

In order to minimize the further formation of mycotoxins after sampling the samples should be submitted for analysis as soon as possible, and should be stored under refrigerated, dry conditions. The first step in mycotoxin analysis is an extraction procedure. Samples are milled and then extracted with a high purity polar organic solvent such as chloroform, acetonitrile, methanol, acetone or ethyl acetate. These solvents are mixed with a given ratio of a more polar solvent (water, dilute acid, aqueous solution of salts) to aid the breaking of weak electrostatic bonds which bind some mycotoxins to other substrate molecules such as proteins. Defatting with petroleum ether or hexane may be done prior to extraction if the subsequent clean-up step is incapable of removing the lipids.

Extraction is followed by clean-up procedure. This step is necessary to eliminate interfering matrix compounds. However, mycotoxins are such a diverse group of chemical compounds that it is difficult to find a simple procedure which specifically removes non-mycotoxin interfering compounds whilst leaving the mycotoxins intact. This is also why it is difficult to find a method for screening a wide range of mycotoxins simultaneously. Clean-up techniques used include: Column- and Thin Layer- Chromatography (TLC); Liquid-liquid Partitioning; Precipitation; Acid-Base Partitioning; Solid Phase Extraction Cartridges; Ion-Exchange Resins; Immuno-affinity Cartridges.

After the clean-up step the extract must be "worked-up" or concentrated to prepare for detection at the low levels required. This is achieved through specialized evaporation equipment and techniques. The extract is then ready for detection and quantification.

As most of the important mycotoxins are fluorescent under ultraviolet light, this method is used in the majority of analytical procedures for mycotoxins. Qualitative assay or plain detection is usually by TLC or mini-column using only a qualitative standard, allowing no more than a semi-quantitative assessment to be made. Fully quantitative determinations require the use of a standard of known concentration, and can be carried out by various techniques including TLC and High Performance Liquid Chromatography (HPLC). Confirmatory tests must be performed if a mycotoxin is thought to

have been detected. Failure to do this could easily lead to false-positive results (interference from another compound). When mycotoxin contamination is identified in a raw material or feed the retained sample (split sample) must be submitted for confirmatory testing by means of TLC or HPLC. These results should then be accepted in any dispute or claim process.

## **LEGAL IMPLICATIONS**

The current regulation governing animal feed in South Africa (Act 36 of 1947 with amendments) only provides limits for Aflatoxins. Other sections referring to the production of safe feed refer specifically to the registration for sale of ingredients or products not provided for in the regulations. Under this scenario a claimant must prove that the product in question caused the alleged damage and that the symptoms are consistent with mycotoxins identified in the feed. It is thus essential that the claimant involve the feed supplier in the sampling of the suspect feed, as should be the case with any suspected feed related problems. Mill retention samples of the allegedly contaminated batch(s) of feed are therefore critical as feed may have become contaminated on farm. The simultaneous occurrence of similar complaints from other customers and in the same or more susceptible species will be important in confirming the contamination of feed prior to delivery. The setting of enforceable, legislated levels for the industry will thus benefit and protect both feed manufacturer and livestock producer.

The main problem is what can be done if a level of mycotoxin contamination is found in an ingredient. Physical and chemical treatments are impractical and of limited success. The opportunity of blending with "clean" material is generally limited due to storage facility constraints at feed mills. The use of binders in the feed is the only practical option, but even with these there appears to be some controversy as to their success other than with aflatoxins. The less polar mycotoxins are a problem, with one option being enzymatic transformation into non-toxic metabolites. The only protection the feed manufacturer may have is to ensure reasonable precautions are in place to prevent mycotoxin contamination. This can only be achieved through the implementation of a sound, practical quality control program together with Good Manufacturing Practice (GMP) procedures.

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