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CONTROL OF AFLATOXIN IN MILK

This bulletin includes technical and latest development on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information on different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **Control of Aflatoxin in Milk**. It may be understood that the information given here is by no means complete.

In this issue …

1. INTRODUCTION

Mycotoxins are secondary metabolites produced by moulds, which grow on food and feedstuffs. Aflatoxin, a type of mycotoxin, is considered to pose the greatest potential hazard to human health and is relevant for milk products. Certain moulds, namely *Aspergillus flavus* and *Aspergillus parasiticus*, which are ubiquitous with spores that are widely disseminated by air currents, produce it.

These moulds frequently grow on plants and seeds – cereal grains, groundnuts, cottonseed, sorghum, almonds, walnuts etc. The proliferation of these moulds, and hence the amount of aflatoxin produced depends on the growth conditions – under stress conditions such as drought, insect infestation or cyclone it is likely to be high. Storage conditions can also lead to aflatoxin contamination after crops have been harvested – hot, humid conditions and damage by insects lead to moulds growing on the food and to high levels of aflatoxin. The existence of mould in a food product, however, does not necessarily signify the presence of aflatoxins. Furthermore, the absence of mould growth on a commodity does not indicate that it is free of aflatoxins, because a toxin can exist after the mould has disappeared.

The presence of aflatoxins in milk is the result of indirect contamination dairy animals consuming aflatoxin B_1 contaminated feedstuffs. Of major importance in this respect is aflatoxin M_1 in milk, which is an animal metabolite of aflatoxin $B_{1.}$

Aflatoxins are highly toxic and carcinogenic. The clinical signs of acute aflatoxicosis include lack of appetite, listlessness, weight loss, neurological abnormalities, jaundice of mucous membranes and convulsions. Death may occur. Other evidence of this condition is gross liver damage through pale colour, other discolouration, necrosis and fat accumulation. Edema in the body cavity and haemorrhaging of the kidneys and intestinal tract may occur.

Aflatoxin B_1 is carcinogenic and aflatoxin M_1 is probably carcinogenic. The carcinogenic potency of aflatoxin M_1 is about 10% only of that of aflatoxin B_1. Codex has established a maximum level of aflatoxin M_1 in milk as 0.5 μg / kg (ppb). It is, therefore, important that necessary preventive measures are taken to control aflatoxin M_1. The Codex Standard *"Code of Practice for the Reduction of Aflatoxin B_1 in Raw Materials and Supplimental Feedingstuffs for Milk Producing Animals"* recommends various measures to be taken at pre- and post-feed manufacturing levels to control the incidence of aflatoxin in feedstuffs. These and some more measures from other references have been extensively mentioned, herein.

This issue of Technews briefly describes the measures to be taken to control the aflatoxin M_1 in milk.

2. TYPES OF AFLATOXINS

Four major types of aflatoxins designated Aflatoxin B_1 [AFB_1], Aflatoxin B_2 [AFB_2], Aflatoxin G_1 [AFG_1] and Aflatoxin G_2 [AFG_2] are currently recognized. Aflatoxin B_1 is generally found in greatest concentration, B_2 and G_2 in least concentration and G_1 at intermediate levels *(Salunkhe et al, 1987).*

AFB_1 and AFB_2 are metabolized in the body of animals to produce two other types of aflatoxins namely AFM_1 and AFM_2, respectively. Another derivative of AFB_1 has been detected and is designated as AFM_4, which co-occurs in certain milks next to AFM_1 *(Van Egmond, 1989).*

3. CLINICAL IMPORTANCE OF AFLATOXINS

The aflatoxins have a very high toxicity and carcinogenecity and elicit a variety of toxic effects in domestic animals and humans.

AFB_1 is the most notorious mycotoxin, feared for its high toxicity and carcinogenic potential. Long-term exposure to low levels of AFB_1 (i.e. 1 ppm) in feed usually leads to terminal liver cancer with mutagenic and teratogenic effects also widely recognized *(Marth and Steele, 1998).*

AFM_1 also is a potent liver carcinogen. However, it appears that AFM_1 possesses only 5% of the liver cancer potential when compared to $AFB_{1.}$ Other studies report that the carcinogenic potential of AFM_1 in humans is likely to be 10 to 100 fold less than that of AFB_1 *(Marth and Steele, 1998).*

Though not much information is available on AFM_4 it is reported to be more toxic and carcinogenic than AFB_1 and AFM_1 *(Van Egmond, 1989).*

4. SOURCE OF AFLATOXIN IN MILK AND MILK PRODUCTS

Aflatoxin contamination of milk and milk products is of two types:

- **Indirect contamination:** Source of aflatoxin in milk and milk products is almost entirely the contaminated feed consumed by the lactating cattle. *A. flavus* and *A. parasiticus* can grow on a variety of agricultural products such as peanuts, cotton seed, corn, wheat, barley, tree nuts, sorghum, fruits etc. and produce aflatoxins in them. When dairy cattle ingest contaminated feed, AFB_1 is metabolized in their body to AFM_1, some of which is then shed in the milk.

- **Direct contamination:** Direct contamination of milk and milk products by the fungi producing aflatoxins is of very little significance. *A. flavus* and *A. parasiticus* do not belong to most frequently species isolated from dairy products *(Marth and Steele, 1998)*. Occasionally other fungal species accidentally contaminating cheeses include other Aspergillus species, which can produce aflatoxins like B_1, B_2, G_1 and G_2 *(IDF, 1997)*. In mould infected cheeses AFB_1 and AFG_1 were detected which diffused at least up to 4 cm. into the cheese from surface during storage *(Marth and Steele, 1998)*. The occurrence of aflatoxins in other dairy products is rare but some of these products may favour growth of aflatoxin producing moulds. Sterilized homogenized milk is a favourable substrate for production of aflatoxins by *A. flavus*. Other products that favour growth of these moulds include cream, unsweetened condensed milk and khoa *(Van Egmond, 1989)*.

5. CARRYOVER RATE OF AFM_1 IN MILK

As stated earlier AFB_1 contaminated feed leads to excretion of AFM_1 in milk, which is recognized as the main aflatoxin in milk.

Excretion of AFM_1 in milk occurs within 12 to 24 hours of ingestion of AFB_1 contaminated feed by the lactating animal. Levels of AFM_1 in milk normally peak within 3 to 6 days and decrease to undetectable levels 2 to 4 days after the exposure of the animal to AFB_1 contaminated feed is stopped *(Marth and Steele, 1998)*. It is reported that carryover rate of AFB_1 from ingested feed to AFM_1 in milk varies from less than 1.0% to 3.0% *(IDF, 1997; Marth & Steele 1998; Van Egmond, 1989)* and in some cases going as high as 6.0% *(IDF, 1997)*.

6. DISTRIBUTION OF AFM_1 IN MILK

AFM_1 is primarily water-soluble. A natural partitioning of AFM_1 occurs during milk separation process into skim milk and cream and then further into butter. Typically the distribution of AFM_1 in skim milk and cream is 90% and 10% respectively. Conversion of cream into butter results in a transfer of up to 2% of the original milk aflatoxin content to the butter and the balance going into buttermilk *(Marth and Steele, 1998).* The AFM_1 is concentrated in skim milk powder and heat concentrated milks. During the manufacture of cheese from AFM_1 contaminated milk, the toxin partitions almost equally between curd and whey, or slightly higher levels in curd due to selective adsorption by casein, resulting in up to 5-8 fold higher content of AFM_1 in cheeses as compared with original levels in cheese milk.

There is no concrete evidence that cold storage, freezing, heat treating, fermenting, concentrating or drying of AFM_1 contaminated milk appreciably destroys its AFM_1 content *(Van Egmond, 1989).*

The stability and activity of AFM_1 in milk or milk products is relatively unaffected by routine dairy processes mentioned above *(Marth and Steele, 1998).*

Little, if any, change in AFM_1 activity has been reported during cheese making or ripening. However, proteolysis of casein during extended storage tends to increase the level of AFM_1 detected *(Marth and Steele, 1998).*

7. CONDITIONS OF MOULD GROWTH AND AFLATOXIN PRODUCTION

Most foodstuffs support the growth of *Aspergillus species* and subsequent production of secondary metabolites like aflatoxins under ideal conditions i.e. warm temperature, high humidity, high moisture content in seeds etc. Factors affecting growth and aflatoxin production by moulds depend upon:

- **Moisture and relative humidity:** Moisture content and relative humidity are the most important environmental factors involved in the growth of the moulds. Moisture requirement depends upon the temperature as well as nutrient available for growth. A minimum moisture requirement of mould spores is lowest at the optimum temperature of growth and is highest near the minimum and maximum temperature for which growth is possible. *A. flavus* has been classified as a mesophyte and has a minimum moisture requirement for growth between 80 and 90 % relative humidity (RH), the minimum for sporulation is 85 % RH *(Salunkhe et al, 1999).*

- **Temperature and Time:** Moulds can grow over a wide range of temperatures. The minimum and maximum temperatures are influenced by other growth conditions such as aeration, moisture, nutrients etc. *A. flavus* is a mesophillic fungus capable of growing between 6 ^{0}C and 46 ^{0}C, the optimum being 36 ^{0}C to 38 ^{0}C *(Salunkhe et al, 1999).* Aflatoxin production is reported to be enhanced when temperatures fluctuate between 5 ^{0}C and 25 ^{0}C. *A. flavus* and *A. parasiticus* fail to produce aflatoxin when grown at temperatures below 7.5 ^{0}C and above 40 ^{0}C.

  The optimal time for aflatoxin production by the moulds depends upon the temperature of growth and the moisture content available. Growth temperatures from 25 ^{0}C to 30 ^{0}C for a period ranging from 7 to 21 days is optimum for aflatoxin production by *A. flavus and A. parasiticus*.

- **Aeration:** Fungi are highly aerobic organisms and need a constant supply of oxygen for growth. Oxygen deficiency has a more marked inhibitory effect on the formation of AFB_1 *(Salunkhe et al, 1999).*

- **Competing flora:** Growth and production of aflatoxin may be affected by the presence of other fungi. Microbial competition between fungi for substrate under favourable conditions will restrict or reduce the amount of aflatoxin formation. Some of the competing micro organisms might restrict or reduce the amount of aflatoxin formation. Some may absorb or degrade the aflatoxin following its formation in the substrate *(Salunkhe et al, 1999).*

8. CONTAMINATION OF AGRICULTURAL COMMODITIES

Mould growth and aflatoxin contamination may occur in agricultural commodities during growing, harvesting, drying, transporting or processing and storage. Environmental conditions such as warm humid conditions, drought condition, insect attack of standing crops, wind velocity and irrigation may effect the growth of fungi and aflatoxin content of different crops. Damaged grains / seeds increase the possibility for direct invasion by the mould and aflatoxin formation due to increased nutrient availability to the infecting fungi. Such damage could be due to insects, mechanical handling or environmental stress conditions like drought etc.

9. CONTROL MEASURES

It has been established that the principal source of AFM_1 in milk is the lactating animal fed on contaminated feed and fodder. Minimizing the level of AFM_1 in milk is almost entirely dependent on careful monitoring of mould growth and AFB_1 level in animal feed. The most effective means to produce AFM_1 free dairy products is to eliminate AFB_1 from feed of the lactating animals. All controllable factors in aflatoxin development should be addressed to reduce the levels in food / feed stuffs.

The control measures for mould growth and aflatoxin production are essentially of two types:

A. Preventive Measures: The prevention of aflatoxin formation on agricultural products is fundamentally a problem of curtailing mould growth which can occur during planting and cultivation, harvesting and drying, storage, transport, processing etc. To ensure a mould free agricultural produce and feed products Codex Alimentarius Commission *(CAC, 1997)* recommends various preventive and control measures to be taken at different stages, which are included in the list given below:

a)Crop production: Important control measures include:

- Apply fertilizers and soil conditioners to assure adequate soil pH and plant nutrition.
- Use of seed varieties developed for fungal resistance etc.
- Sow and harvest crops timely to avoid high temperature and draught stress during the period of seed development.
- Avoid plant stress by adopting good agronomic practices like adequate irrigation, avoidance of overcrowding of plants, elimination of weeds and fungal vectors in the growing crops and adopting crop rotation.
- Minimize insect damage and fungal infections by use of appropriate insecticides and fungicides.
- Minimize mechanical damage to crops during cultivation.

b)Harvesting: Important control measures include:

- Harvest crops at full

maturity if such a measure would not subject it to extreme heat, rainfall or draught conditions.

- Avoid mechanical injury during harvesting.
- Carefully and quickly dry crops to a minimum moisture content (7 to 8%) etc.
- Avoid piling or heaping wet freshly harvested commodities for more than a few hours prior to drying or thrashing to lessen risk of fungal growth.
- Ensure adequate protection from rain during sun drying.

c)Storage: Prevention of attack by fungi and insects and pests is of prime importance in post harvest stage. Important control measures include:

- Maintain good sanitation and proper storage conditions (dry and well ventilated) for storage structures.
- Use clean and dry bags and ensure stacking on pallets.
- Prevent insect infestation by the use of approved insecticides.
- Prevent access by rodents and birds.
- Store at as low a temperature as possible and maintain proper moisture conditions (RH 70% max.) through continuous circulation of air.
- Before the start of the season, and periodically thereafter, the storage structure should be thoroughly cleaned and swept down, and all trash, spillage and grains, which are thus collected, should be burnt, buried or removed far away.
- Bureau of Indian standards *(BIS, 1979)* recommends that the area and walls should be thoroughly sprayed with an emulsified residual insecticide, such as 0.3 % lindane, or 3.0 % malathion at the rate of 5 litres per 100 m^{2}.
- Use of appropriate fungicides ammonia-phosphine (50mg each per litre) or propionic acid (1%) is effective in killing moulds and preventing aflatoxin production *(Salunkhe et al, 1999)*

d)Transport: Important control measures include:

- Ensure periodic disinfestations of transport vehicles and containers with approved fumigants.

- Use tarpaulins and airtight container to protect the material from moisture absorption during transit.
- Avoid insect and rodent infestation during shipment by use of insect proof containers.

e)Animal feed manufacturing: It is important to:

- Use only those agricultural products in animal feed manufacturing, which are low in aflatoxin B_1 content.
  Aflatoxin level of 20 μg / kg of animal feed i.e. 20 ppb (United States requirement), if observed, will consistently yield acceptable milk containing less than 0.5 μg / litre of milk i.e. 0.5 ppb *(Marth & Steele, 1998)*
- Ensure that the equipment used in feed manufacturing is kept clean, free of dust and feed accumulation
- Ensure proper storage of feed material as explained earlier

B. Detoxification Measures: Prevention of aflatoxin contamination of foods and feedstuffs is not always possible. Under certain environmental conditions mould infestation and aflatoxin contamination is inevitable. In such cases it is important to remove or destroy the toxin present in the feedstuff before manufacturing animal feed. Removal or inactivation of toxins is achieved through well-established physical, chemical or microbiological processes.

Aflatoxins can be removed from the food grains chemically by i) Extraction with mixed solvent Azeotropes, ii) Extraction with Chloroform, iii) Extraction with Aqueous Acetone or iv) Extraction with calcium chloride and other solvents *(Salunkhe et al, 1999).*

Aflatoxins can also be inactivated in the food grains by chemical treatments with i) Ammonia, ii) Sodium bisulphate (0.5 to 1.0 %) iii) Hydrogen peroxide and various other chemicals *(Salunkhe et al, 1999).*

C. Detoxification of Milk: Though not permitted legally as yet treatment of milk with peroxides, riboflavin, lactoperoxidase, sulphites and bisulphites, UV irradiation and certain particulate matter such as bentonite has proven effective in experimentally reducing levels of aflatoxin M_1 in contaminated milk.

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