

Executive Summary

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**Title: Survey of Aflatoxins in Cattle feed and Milk from Tamil Nadu by ELISA
Technique and Control Measures**

Introduction and Scope:

Aflatoxins are one of the most potent toxic secondary metabolites secreted by fungi named as *Aspergillus flavus* which are commonly included in mycotoxins that occur naturally in various agricultural food commodities and other nutritious plant products. Aflatoxicosis is poisoning of food and feed materials that result from ingestion of aflatoxins in contaminated food or feed to animals and human which produce serious health hazards. Aflatoxin poisoning is reported from all parts of world in human and animals like cattle, horses, rabbits, poultry and other non-human primates. These aflatoxin producing fungi grow and can contaminate a wide variety of agricultural commodities including cereal grains, pulses, oilseeds, spices and condiments which is also passed to mixed feed and formulated food products through various by-products which leading to animal and cause hepatocancers in human especially children and veterinary. Aflatoxins refer to a group of several chemicals produced by the fungus *Aspergillus flavus* and related species in which the most common aflatoxins are B1, B2, G1, G2 generally occurred from contaminated food and feeds and the aflatoxin M1 and M2 are found in milk which is metabolized normally from aflatoxin B1 through feed although other conversions may occur. Aflatoxin B1 and M1 are leading to deleterious effect to human and animals if ingested through contaminated food and feed more than the allowable limit as more than 20 ppb depending on the Food and Drug Administration (FDI) standards of various countries. Aflatoxin M1 has been shown to be hepatocarcinogenic at 50ppb in Fischer male rats with the potency of 2–10% of that of AFB1 and also induces low incidences of intestinal adenocarcinomas.

The contamination of aflatoxins by the infestation of *Aspergillus flavus* in many food commodities account per annum for millions of dollars in losses worldwide specially in tropical countries like India. Today aflatoxin has been one of the most important global

concerns regarding contamination of food products. Diet is the major way through which humans as well as animals are exposed to aflatoxins. Taking into account the development in Codex alimentarius, recently EC has introduced the maximum accepted/residue levels for aflatoxin in animal feeds as 0.02 mg/kg, i.e., 20 ppb in all feed materials and in the most complete and complementary feedstuffs for cattle, sheep, goats, pigs and poultry, while it is 0.005 mg/kg in complete feeding stuffs for dairy animals and 0.01 mg/kg for complete feeding stuffs for calves and lambs. The raw materials such as rice bran, oilseed cakes and pulses used for formulation of cattle feed support the growth and production of aflatoxin by toxigenic fungi known as *Aspergillus flavus*.

Based on the above scientific background, the present research project has been carried out to find the present status of contamination of aflatoxins in a large number of cattle feed and milk samples from Tamil Nadu. The survey of aflatoxin B₁ contamination in various animal feed samples and the level of occurrence of aflatoxin M₁ in different milk samples from the market of Tamil Nadu were considered significant and the study was undertaken. If the level of aflatoxins is higher than the permissible level from the above survey results of the present study, then the preventive and control measures also planned by various possible ways.

Objectives

- To Study the natural occurrence of aflatoxin B₁ in large number of Cattle-feed samples collected from markets and individuals of Tamil Nadu.
- To study the occurrence of toxigenic *Aspergillus flavus* in the above cattle feed samples collected from above sources.
- To study the contamination of storage fungi in the above cattle feed samples. (i.e. the species of *Aspergillus* and *Penicillium* which are growing in agricultural commodities during maturity and storage).
- To Study the natural occurrence of aflatoxin M₁ in Milk samples collected from markets, individuals and farms of Tamil Nadu.
- If the level of contamination of aflatoxin is high, then the study of control measures for aflatoxin B₁ and M₁ milk through physical, chemical and biological methods.

Methodology

1. Field visit and collection of cattle feed samples from different parts of Tamil Nadu.
2. Determination of Moisture Content of feed samples by Hot Air Oven drying method.
3. Analysis of storage fungi in cattle feed samples by serial dilution method and testing of toxigenicity of *Aspergillus flavus* isolated from feed samples.
4. Aflatoxin Analysis in cattle feed samples by TLC and ELISA method.
5. Field visit and collection of Milk samples from different parts of Tamil Nadu.
6. Aflatoxin Analysis in milk samples by TLC method.

Results

Field visit and Sample collection: Totally, 150 cattle feed and 100 milk samples were collected from local markets, individuals and dairy farms in Chennai, Kancheepuram, Thiruvallur, Villupuram, Thiruvannamalai, Vellore, Namakkal, Salem, Erode and Coimbatore districts of Tamil Nadu. The cattle feed samples were appeared in the form of compressed and dried pellets in various lengths of 1 cm to 3 cm with 0.5 cm breadth except very few are available in powder form. Milk samples collected were in the form of liquid available in the plastic sachet in the markets and from the individuals.

Moisture content in cattle feed samples: The moisture content of the cattle feed samples varied from 5.6% to 15.0% with an average of 9.0%. The moisture content of the cattle feed samples may play a role on the growth of different species of storage fungi such as *Aspergillus* and *Penicillium* which may be responsible for production of toxins during further storage.

Storage fungi and aflatoxigenic fungi in cattle feed samples: Totally 50 samples were randomly analysed for the occurrence with storage fungi including aflatoxigenic *Aspergillus flavus*. The storage fungi mainly comprising different species of *Aspergillus* and *Penicillium* encountered in cattle feed samples in varying numbers. The individual species of storage fungi observed were *Aspergillus niger*, *A. glaucus*, *A. flavus*, *A. terreus*, *A. nidulans*, *A. fumigates*, *A. candidus*, *Penicillium citrinum*, *P. funiculosum*, *P. chrysogenum*, *P. tardum*, *Mucor mucedo*, *Rhizopus stolonifer*, *Pyricularia oryzae*, and nonsporulated white mycelial fungi. Few other fungi like *Mucor*, *Rhizopus*, *Fusarium*, *Alternaria*, *Pyricularia*, *Helminthosporium* and *cladosporium* also were observed in very less numbers in cattle feed samples. The overall population of storage fungi in individual cattle feed samples is observed

as in the range of 300 to 3,600 cfu/g with an average of 1220 cfu/g. Among the above species of fungi, *Aspergillus glaucus* observed as very frequently and dominant mycoflora in the range of 200 to 2000 cfu/g with an average of 856 cfu/g. followed by *A. terreus*, *Penicillium citrinum* and *A. fumigatus*. Out of 50 cattle feed samples analysed for the storage fungi, 21 samples were found contaminated with of *A.flavus* in the range of 100 cfu/g to 600 cfu/g with an average of 250 cfu/g.. Out of 21 strains of *A. flavus*, only 11 were found to produce aflatoxins B₁ i.e 52% of *A.flavus* strain found to be toxigenic.

Aflatoxin B₁ in cattle feed samples: Out of 150 cattle feed samples analysed, 128 samples found with positive for the presence of aflatoxin B₁ and 22 samples were negative for aflatoxin B₁ contamination. The concentration of aflatoxin B₁ in different cattle feed samples varying in the range from 2 ppb to 180 ppb with an average of 20 ppb. Of the 150 samples from various brands of cattle feed analysed, the samples from all the brands show positive for aflatoxin B₁ at various level of concentration. However, the cattle feed samples from the brands found with aflatoxin B₁ also observed with negative for the presence of aflatoxin B₁. These differences might be due to the presence aflatoxin B₁contamination in raw materials used for formulation of cattle feed and the processing parameters. The lower concentration or absence of aflatoxin B₁ in cattle feed samples (possibly) indicate the yield of lower level of aflatoxin M₁ occurrence in milk samples due to the metabolic degradation lactating animals.

Aflatoxin M₁ in Milk samples: Out of 100 milk samples analysed for the presence of aflatoxin, 12 samples observed to positive results for aflatoxin M₁ and 88 samples were free of contamination. The concentration of aflatoxin M₁ in different milk samples varying in the range from 2 to 20 ppb with an average of 6 ppb. Of the 100 milk samples from 5 brands and different individual farm samples analysed, the samples from few sources showed positive for aflatoxin M₁. The lower concentration or absence of aflatoxin M₁ in milk samples (possibly) will indicate the feed consumed by cow contain lesser amount of aflatoxin B₁ which metabolized to yield aflatoxin M₁ with lesser level in the milk.

Conclusion:

It is concluded that from the present study as the natural occurrence of aflatoxin B₁ in most of the cattle feed samples collected from different places of Tamil Nadu were with lower concentration as below the permissible limit (80 ppb) which is safer level except very few samples (5% of samples). This may indicates the processing of cattle feed samples during formulation with different raw materials including agricultural commodities might be involved with stringent detoxification methods. The reports from various research laboratories show that the aflatoxin detoxification methods such as physical, chemical and

biological methods drastically reduce the aflatoxin concentration in food and feed materials. The lower moisture percentage observed in the cattle feed samples indicate the raw materials with low moisture and further moisture removal during the processing which prevent further contamination of fungi and toxins. The population of storage fungi including *Aspergillus* and *Pencillium* species in cattle feed samples were observed to be less when compared with agricultural commodities and its bi-products used for formulation of cattle feed which might be due to killing of during feed processing. The remarkable finding of the present study is the lower incidence of aflatoxin producing *Aspergillus flavus* in cattle feed samples, which indicate the further contamination is seldom possible even during storage of cattle feed. Another important observation is the constant occurrence of *Aspergillus glaucus* in almost all the feed samples which might be due to the heat tolerance and osmophilic nature in the processing environment. It is also concluded that most of the cattle feed samples available in the market is contaminated with permissible limit of aflatoxin B₁ which is possible to occur in lower level after metabolic degradation in cow and secretion of milk with safer level of aflatoxin M₁. The occurrence of aflatoxin M₁ in most of the milk samples collected from local market and field of Tamil Nadu found almost absence of aflatoxin as well as with very lower concentration as below the permissible limit as 20 ppb found to be safer level for human consumption except very few samples (1% of samples). The lower concentration of aflatoxin M₁ in milk samples reflect the occurrence of lower amount of afltoxin B₁ in cattle-feed samples and indicate appropriate quantity yield during metabolism and conversions. Overall, the present study conclude that as the majority of cattle feed samples and milk samples available from the market appeared to be with in the permissible limit (safer level) of aflatoxin contamination.