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Occurrence of mycotoxins in livestock feeds and feed stuffs of Tamil Nadu

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Abstract

The livestock feed and feed ingredients were screened for the presence of aflatoxin B1, citrinin, penicillic Acid, T2, ochratoxin A and zearalenone. The samples were collected from different livestock farmers/farms of Tamil Nadu. Mycotoxins were determined in all the samples. The present study clearly indicates high occurrence of citrinin highly predominant followed by Aflatoxin B1 and ochratoxin A in feedstuffs and feeds. Aflatoxins B1, citrinin, ochratoxin A were the most common mycotoxins observed. The aflatoxin B1 levels ranged between 50 to 80 µg kg^{-1}, ochratoxin A levels ranged between 20 to 160 µg kg^{-1}, Citrinin levels ranged between 20 to 350 µg kg^{-1}, penicillic acid levels ranged between 20 to 30 µg kg^{-1}, T2 Toxin levels ranged between 75 to 450 µg kg^{-1} and zearalenone levels ranged between 150 to 1000 µg kg^{-1} respectively. The results of the study warrant the need for sustained monitoring of these commodities periodically and evolve policies which discourage the marketing of toxin contaminated feeds as existing in the developed countries.

Key words

Mycotoxins analysis, Aflatoxin B1, Livestock feed

Introduction

Mycotoxins are structurally diverse fungal metabolites produced by fungi, not essential to fungal growth and produced periodically under fungal stress. They can contaminate a variety of mixed feed and food leading to nutrient losses and producing adverse effects on animal and human health. Specific competent species like *Aspergillus*, *Fusarium* and *Penicillium* are able to produce these toxins that account per annum for millions of dollars in losses world-wide in condemned agricultural products (Bouhet and Oswald, 2005).

Fungal metabolites are generally associated with fungi belonging to the genera *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*. Toxigenic *Alternia* and *Fusarium* species are often classified as field fungi, while *Aspergillus* and *Penicillium* species are considered storage fungi. Although more than 100 mycotoxins have been identified, less than 10 are of concern due to their natural occurrence and toxicity. The most common *Fusarium* mycotoxins are trichothecenes, zearalenone and fumonisins. The mycotoxins of interest produced by *Aspergillus* spp. include aflatoxins (AFLA) and ochratoxin A (OTA), while *Penicillium* spp. produces ochratoxin A, citrinin and patulin among the more important mycotoxins (Logrieco et *al*., 2003).

However, not all fungal growth results in mycotoxin formation and detection of fungi does not imply necessarily the presence of mycotoxins (Morgavi and Riley, 2007). Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic effects resulting in teratogenic, carcinogenic, and oestrogenic or immune-suppressive effects (Pestka, 2007). Direct consequences of consumption of mycotoxin animal-feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain. Increased disease incidence (due to immune-suppression), and reduced reproductive capacities which leads to economic losses (Phakamile *et al*., 2008).

Due to modern laboratory methods and a growing interest in this field of research, more than 300 different

mycotoxins have been differentiated so far. However for a practical consideration in the feed manufacturing process only a small number of toxins are of relevance with aflatoxin B1, trichothecenes, zearalenone, ochratoxin A and fumonisin being of particular interest, although it has to be mentioned that the extent of harm each toxin (group) can cause is highly species-dependant (Binder *et al.,* 2007).

The objective of the present study was to report the prevalence of mycotoxins in feedstuffs and livestock feeds relevant to the livestock farmers and feed industry.

Materials and Methods

A total number of 441 feed stuff (18 varieties) and 475 feed samples (21 varieties) collected from several feed mills and livestock farms from Tamil Nadu. A minimum of 1kg of sample was collected in cloth bags free from moisture were appropriately labelled with lab unique ID. All the feed samples were separately ground and 25gm aliquots weighed before extraction. All reagents were of analytical grade (Emerck). The mycotoxin standards, aflatoxin B1, citrinin, penicillic acid, ochratoxin A, zearalenone and T2 toxin were obtained from LGC Promochem, U.K. These standards were calibrated and checked for its purity by UV Spectrophotometer (AOAC, 2000). The mycotoxin standards solutions used in the study were dissolved in specific solvents : Aflatoxin B1 1µg ml^{-1} in benzene: acetonitrile (98:2); ochratoxin A 1µg ml^{-1} in benzene acetic acid (9:1); citrinin 5µg ml^{-1} in chloroform; T2 10µg ml^{-1} in chloroform; zearalenone 10µg ml^{-1} in chloroform, penicillic acid 10µg ml^{-1} in chloroform.

Samples were finally ground (20 mesh particle size) using explosion proof laboratory blender. All the samples were simultaneously analyzed for aflatoxin B1 by Romers all purpose method (Romer, 1975), while Ochratoxin A, zearalenone, penicillic acid, citrinin and T2 toxin were analyzed using multi mycotoxin screening method (Scott *et al.,* 1972). The samples after extraction, cleanup, concentrated dried were subjected to thin layer chromatography. Thin layer chromatography of mycotoxins and extracted samples were performed on a preheated silica gel plate (E.Merck) and developed in toluene, ethyl acetate and 90% formic acid (6:3:1,V/V/V"). Toxins were estimated by visual comparison with known amounts of standard spotted on the same TLC Plates. The presence of mycotoxins were identified by their fluorescence property and confirmed chemically and then quantified (Scott *et al*., 1972). All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

For the purpose of data analysis, non-detect levels are based on the detection limits of the test method for each toxin; Aflatoxins <5µg kg^{-1}; Zearalenone <50µg kg^{-1}; Citrinin <20µg kg^{-1}; Ochratoxin A <10 µg kg^{-1}; T2 Toxin <50µg kg^{-1} and Penicillic Acid <50µg kg^{-1}.

Method validation was performed in blank Feed samples spiked with mycotoxin standards at Aflatoxin 5µg kg^{-1}; Zearalenone 50µg kg^{-1}; Citrinin 20µg kg^{-1}; Ochratoxin A 10µg kg^{-1}; T2 Toxin 50µg kg^{-1} and Penicillic Acid 50µg kg^{-1}. The average recovery rates were above 85%.

Results and Discussion

The analysis of mycotoxin showed that Aflatoxin B_1 contaminated most of the feed stuff and feeds. Out of 441 feed stuff samples analyzed, aflatoxin B_1 was detected in 232 samples, ochratoxin A in 102 samples, citrinin in 221 samples, penicillic acid in 13 samples, T_2 toxin in 16 and zearalenone in 86 samples. Similarly, out of 475 feed samples analyzed for mycotoxins, aflatoxin B_1 were detected in 246 samples, ochratoxin in 35 samples, citrinin in 132 samples, penicillic acid in 3 samples, T_2 toxin in 59 and zearalenone in 33 samples, respectively. The range of the above mentioned mycotoxins in feed stuff and feed are given in Table 1 and 2.

Among the mycotoxins, Aflatoxin B1 levels in dairy cattle feed exceeded the permissible limit (20 µg kg^{-1}) of European Union. Aflatoxin B1 level in dairy feed and food stuff are important to human health since approximately 1 to 2% of the aflatoxin B1 in the animal feed is transformed to aflatoxin M1 in milk. Therefore, aflatoxin B1 concentration in feed above 20 µg kg^{-1} may result in milk containing higher Aflatoxin M1 (Oruc *et al.,* 2007)

The results obtained in the present study indicate high risk for human health because of the possibility of indirect exposure through meat and other animal products. It has been estimated that 25% of the world's crops may be contaminated with mycotoxins and the worldwide contamination of foods and feeds with mycotoxins is a significant problem. Since 1994 many countries have developed regulations for aflatoxin, ochratoxin A and deoxynivalenol in animal feeds and human food but the regulation may vary from country to country. The European Union has set a maximum level of Aflatoxin in agriculture commodities with aflatoxine B_1 of 4µg kg^{-1} whereas in US, the Federal Food Drug and Cosmetic Act has regulated 20 µg kg^{-1} aflatoxin in foods and feeds (Binder, 2007).

The types and levels of mycotoxins in raw materials and finished feeds depend on various factors such as the geographical area, weather, type of grains, birds and insects damage to corn (Sutton,1984), storage conditions, milling practice (Young *et al.*, 1984) and farm management. Sampling of samples is also important as the integrity of test result produced will be determined by the effectiveness of sampling, sample preparation and analysis step (Jackie Linden, 2006). Since the farmer's greatest concern is on the adverse effect of mycotoxins against their animal productivity, finish feed will be more accurate a sample for mycotoxin testing.

Table 1 : Concentration (µg kg^{-1}) of various mycotoxins in different feedstuffs

Feedstuffs	N	Aflatoxin B1		Ochratoxin A		Citrinin		Penicillic Acid		T2		Zearalenone	
		n*	Content	n*	Content	n*	Content	n*	Content	n*	Content	n*	Content
Maize	123	60	75±1.5	18	45±2.5	110	350±40.5	10	30±2.5	12	100±5.5	25	1000±150.1
Bajra	29	10	40±2.5	5	40±2.5	10	150±10.3	-	-	-	-	16	750±25.2
Soya	17	5	40±2	8	80±3.5	4	80±10.1	-	-	-	-	-	-
Sunflower oil cake	16	10	50±2.5	10	160±8.5	5	40±5.5	-	-	-	-	10	300±15.2
Wheat bran	33	22	30±2.2	5	40±2.5	16	60±10.2	-	-	-	-	10	400±25.1
Deoiled rice bran	43	35	40±2.5	20	60±5.5	10	75±10.3	-	-	-	-	-	-
Rape seed	13	8	20±2.5	3	40±5.1	-	5 50±2.5	-	-	-	-	-	-
Fish meal	17	5	25±2.5	-	-	-	-	-	-	-	-	-	-
Groundnut oil cake	17	15	80±5.5	8	60±4.5	5	65±2.5	3	20±1.6	-	-	-	-
Sesame oil cake	13	6	20±2.5	3	45±2.2	5	55±2.5	-	-	-	-	-	-
Broken rice	11	6	50±2.5	5	40±6.1	10	75±10.2	-	-	-	-	5	250±10.3
Ragi	26	12	40±2.5	2	40±1.2	11	60±10.3	-	-	-	-	-	-
Sorghum mash	22	10	60±2.5	5	60±2.5	10	40±2.5	-	-	-	-	5	500±25.2
Barley	19	10	40±3.5	10	40±6.5	10	65±10.1	-	-	4	75±10.1	5	400±10.5
Oats	16	5	45±2.5	-	-	10	60±5	-	-	-	-	-	-
Cotton seed cake	5	3	50±5.5	-	-	-	-	-	-	-	-	-	-
Horse gram	9	4	35±3.5	-	-	-	-	-	-	-	-	-	-
Mustard oil cake	12	6	15±4.5	-	-	-	-	-	-	-	-	-	-

N = total of samples n* = positive sample (-) = not detected. Values are mean of three replicates ± SD

Table 2 : Concentration (µg kg^{-1}) of various mycotoxins in different feeds

Feeds	N	Aflatoxin B1		Ochratoxin A		Citrinin		Penicillic Acid		T2		Zearalenonen	
		n*	content	n*	content	n*	content	n*	content	n*	content	n*	content
Chick mash	13	7	20±1.5	2	40±2.5	3	40±10.1	-	-		-	-	-
Broiler mash	11	3	25±2.3	1	40±2.3	5	20±1.5	-	-	4	450±10.2	-	-
Grower mash	13	7	40±3.3	1	40±2.5	2	40±5.1	-	-	5	350±10.2	-	-
Layer mash	30	15	45±3.5	6	60±2.5	10	80±1.3	3	20±3.5	7	350±10.2	-	-
Broiler starter	43	19	40±3.1	4	60±3.5	16	40±2.5	-	-		-	-	-
Broiler finisher	58	32	50±2.5	10	60±3.6	16	50±2.5	-	-	15	125±15.1	-	-
Broiler breeder	16	9	30±2.6	-	-	7	45±10.3	-	-	5	250±10.1	-	-
Layer breeder	17	7	45±4.5	-	-	6	55±2.5	-	-		-	-	-
Calf starter	36	18	35±2.1	-	-	12	60±2.1	-	-		-	-	-
Pre layer feed	11	8	30±2.4	-	-	2	40±2.2	-	-	4	125±10.1	6	250±10.2
Pre layer mash	15	5	40±2.6	-	-	-	-	-	-	6	200±10.1	-	-
Cattle pellet feed	26	16	40±1.5	-	-	10	60±2.5	-	-	-	-	-	-
Young layer feed	40	20	45±2.5	3	60±2.5	6	45±2.1	-	-	7	350±20.5	5	150±10.3
Layer grower	17	12	40±5.1	-	-	7	55±2.6	-	-	6	350±25.5	6	250±15.4
Layer starter crumble	16	6	45±2.5	-	-	4	45±2.1	-	-	-	-	-	-
Layer pre layer crumble	15	10	40±10	-	-	4	55±2.6	-	-	-	-	6	150±5.5
Rabbit feed	10	5	50±3.5	-	-	1	55±2.5	-	-	-	-	-	-
Cattle feed	40	23	60±3.5	4	40±2.6	13	60±3.1	-	-	-	-	10	500±10
Bull feed	13	7	35±2.7	2	20±3.5	-	-	-	-	-	-	-	-
J.Quail starter mash	8	2	45±2.5	2	40±2.5	8	40±2.5	-	-	-	-	-	-
Sheep feed	27	15	45±3.6	-	-	-	-	-	-	-	-	-	-

N = total of samples, n* = positive sample, (-) = not detected. Values are mean of triplicate ± SD

During 2004 and 2005, a survey was conducted to study the incidences of aflatoxin, ochratoxin and T-2 toxin in various feed ingredients and finished feeds collected from different states of the country. Out of 441 samples analyzed , 824 samples were found to be positive for the presence of aflatoxin, ochratoxin and T-2 toxin (Devegowda *et al.*, 2005). Of these cereals, cereal by-products, oilseed meals and finished feeds were tested positive for mycotoxins. The authors reiterated that not only aflatoxins but ochratoxin and T-2 toxin are also a problem in the region.

Our findings are in agreement with earlier reports (Alkhalaf *et al.,* 2010; Muhammad Kashif Saleemi *et al*., 2010) where aflatoxin B1 was found to be widely distributed in feed stuff. A vast majority of outbreaks in farm animals have been caused by aflatoxin, fumonisins and zearalenone and to a lesser can occur even at low levels of exposure to mycotoxins in feed. A combination of mycotoxins may pose a greater production loss than individual mycotoxins seperately. The economic losses have been associated in terms of reduced productivity, such as lowered egg production, reproductive effects, susceptability to infections resulting in increased morbidity and finally mortality. Exposure of farm animals to mycotoxins through animal feed have in the past resulted in field outbreaks.

Multi-toxin occurrence may be one important explanation for divergences in effect-levels described in the scientific literature, where mostly purified mycotoxins are used in majority of studies. In field outbreaks, naturally contaminated feeds may contain multiple mycotoxins and thus apparently lower contamination levels of single specific mycotoxins can be associated with more severe effects. Poor performance in food animals have varied primary etiologies which need to be diagnosed by field veterinarians. Mycotoxicosis needs to be ruled out as one primary cause (Khan *et al.,* 2005). This study is in concurrence with many studies which indicate that mycotoxins in raw materials and finished feed are common. The results obtained in the study suggest high risk for human health because of the possibility of indirect exposure through meat and other animal products (Beg *et al.,* 2006). Producers need to be attentive to raw materials quality control and should take positive actions to control mould growth and should use mycotoxin binders which are proven effective against the various mycotoxins to be included in animal feeds (Casarin, 2000). In addition to protect the animal and human health, government agencies need to inform both farmers and livestock industries about the importance of the mycotoxin. Factors influencing mycotoxin production in feedstuffs have to be clearly defined and preventive measures should be taken to decrease the risk of mycotoxin and its consequences in livestock products meant for human consumption (Miraglia *et al.,* 2010).

In conclusion the observations made in our study and recent reports in literature extensively support the natural co-occurrence of aflatoxins, ochratoxin A, citrinin, T2 and zearalenone in feeds and feeds stuffs (Devegowda *et al.,* 2005; Martins *et al.,* 2008; Marcela Beatriz roige *et al.,* 2009; Alkhalaf *et al.,* 2010; Muhammad Kashif Saleemi *et al.,* 2010). This warrants the need for an expanding monitoring programme to generate database on the suspected mycotoxins and establish regulatory permissible levels for setting up of mycotoxin safety standards. This will help in identifying the source of contamination executing control measures, enabling better risk assessment by proper and providing ecnonmic benefits.

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