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MYCOTOXIN CONTAMINATION IN FEED INGREDIENTS AND THEIR IMPLICATION ON LIVESTOCK HEALTH AND PRODUCTION

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Fungal contamination of foods and feeds is not a new phenomenon; it has been well established since biblical times. Mycotoxins are secondary metabolites produced by a wide range of fungi, mainly mold. There are more than one hundred species of molds that produce mycotoxins; the three most important species are Aspergillus, Penicillium and Fusarium. Nearly three hundred types of mycotoxins have been recorded so far. Livestock and birds consuming feeds contaminated with mycotoxins suffer from mycotoxicosis. Though the problem has been known for several centuries, until the early sixties it was mainly thought in terms of toxicity to animals and birds and the consequent economic loss (Cheeke and Shull, 1985).

Classification of mycotoxins based on their biological effects:

Ingestion of mycotoxin contaminated feed by livestock and poultry cause variable effects which ranges from a drop in production/ decrease in growth rate to mortality, depending on the degree of contamination. The toxic effects of these mycotoxins vary according to the type of toxin involved. Based on the biological effects to the livestock and poultry the toxins have been classified below (adapted form Cheeke and Shull, 1985).

Hepatotoxins:- Aflatoxin, Sporidesmin, RubratoxinB, Sterigmatocystin, Tricothecenes, Ochratoxin A, PhomposinA, Cyclopiazonic Acid

Nephrotoxins:- OchratoxinA, Citrinin, Aflatoxin, Oosporin, Cyclopiazonic Acid, Stearigmatocystin

Neurotoxins:- Trichothecenes (vomitoxin, satratoxin), Salframine, PenitremA, Ergot alkaloids, Ochratoxin A

Genitoxins:- Zearalenone, Ergot alkaloids, Aflatoxins (mostly ruminants)

Dermitoxins:- 12,13-Epoxytrichothecenes (T2 toxin, nivalenol)

Carcinogens: - Aflatoxin, Sterigmatocystin, Luteoskyrin, Patulin, and Penicilic acid, T2, OchratoxinA and Citrinin

Teratogenes:- Aflatoxin. Ochratoxin

Immunosuppressents:- Aflatoxin, T2, Ochratoxin, Citrinin, Oosporin

Hematological agents:- Aflatoxin, Trichothecenes.

Modified Mario.O.Tapia Method For Estimating Multi-Mycotoxins In Feed/Raw Materials

The multitoxin is extracted with acetonitrile, potassium chloride, filtered using hydrochloric acid and defatted twice with hexane. The fat free extract is further extracted using chloroform. This is dried and diluted in highly pure chloroform and spotted on a pre-coated Aluminium Merck sheet of 0.25nm thickness, along with known standards. The plate is allowed to air dry and developed in chloroform and acetone on one direction and Toluene, Ethyl acetate and Formic acid in the second direction perpendicular to the first. The plates are dried and quantified under the long-wave of UV light.

Mycotoxins Recorded in our laboratory:

In Namakkal with the layer population reaching nearly two crores the daily requirement for feed is nearly 2500 tonnes and an equal quantity of feed is needed for the broilers in the adjacent Coimbatore district. Nearly 95% of the raw materials are procured from across India. Further, we receive samples for testing from almost all the states in the country, hence the results obtained in this laboratory can be taken as an indication of the toxin contamination of feed and ingredients in India.

Aflatoxin: Aflatoxin is the most prevalent of all the mycotoxins. A total of 31307 samples were screened in this laboratory from May 1994 to December 2001 for the contamination of aflatoxins, of which 58% were oilcakes, 27% were feed and 15% were cereals. The bulk of the positive samples were de-oiled groundnut cake (DOGNC) and maize.

Oil Cakes: Out of the 18290 samples of oil cakes

analysed DOGNC was the major component (7882). Invariably all the DOGNC samples (99.1%, Table 1) were found to be contaminated, in 48% of the samples the level was below 100 ppb, but in 46% samples it ranged between 100 and 500 ppb and 5% of the samples the level was above 500ppb. Of the 5717 samples of sunflower cake (SFOC, Table 2) samples screened 17% were negative for AFTB1 in 63% of the samples the level of toxin ranged between 1 and 20 ppb , while in 17% of the samples the levels of the toxin ranged from 21 to 100ppb and only in 2% of the samples the levels were above 100ppb. Soyabean meal (SBM) was the third predominant oil cake subjected for aflatoxin analysis, 84.3% of the meal screened were negative for aflatoxins. Further, 15.2% recorded below 20ppb of AFTB1, and in only 0.5% of the samples the level exceeded 20ppb. Reviewing the influence of season on the toxin contamination of the samples, it was found that even during non rainy season nearly 99% of DOGNC, 70% of SFOC and 36% of SBM were positive for the presence of AFTB1.

Cereals: Maize was the predominant cereal screened (85%), nearly 56% (Table 3) of the maize samples were negative and in 22% the level of AFTB1 was below 20ppb. In 12% of the maize samples the level ranged between 21 and 100ppb, 4% of the samples the level was between 101 and 500 ppb, in 1% of the samples the level was above 500ppb, studying the influence of the season it was found that nearly 35% of the maize samples tested in the non-rainy season were found to be contaminated with AFTB1. Among the other cereals tested 65% of jowar and 39% of bajra were positive for the presence of AFTB1. In Jowar the majority (55%) contained below 20ppb and only 10% contained levels above 20ppb of AFTB1, 8.2% contained between 21 and 100ppb and only 1.8% contained above 100ppb of AFTB1. In bajra only 1.5% contained levels above 20ppb of AFTB1.

Feeds: Only 23% of the8387 feed samples (Table 4) screened were negative for AFTB1. In 51% of the samples the level was below 20ppb. In 21% of the samples the level ranged from 21 to 100ppb, in 5% of the samples it was between 100 and 500ppb, in 0.4% it was above 500ppb. The effect of the season followed the pattern of the oil cakes, nearly 71% of the feed samples were positive for AFTB1 during the non-rainy season.

Mycotoxins other than aflatoxins

A 2-Dimensional thin layer chromatographic method was modified and standardized in this laboratory from an earlier procedure (Tapia, 1985). Quantification of ochratoxin A (OA), citrinin, T2-toxin, zearalenone, sterigmatocystin, aflatoxins was done. A total of 4570 samples were received for analysis between April 1997 and December 2001. While 2769 samples were oil cakes, 1405 samples were feed and 379 was maize. OA, citrinin and aflatoxin were the mycotoxins predominantly observed in the feed ingredients and feeds.

Ochratoxin: Seventy eight per cent of SFOC samples were found to be contaminated with OA. While 55.3% samples contained up to 100 ppb, 22.8% contained levels higher than 100ppb and 63.6% samples of SFOC were co-contaminated with AFTB1. Out of 106 samples of DOGNC positive for OA 77% contained varying levels of AFTB1. Of the 1405 feed samples, 146 samples were positive for OA. In 132 samples, co-contamination with aflatoxin was observed. Very little contamination was observed in SBM and rapeseed meal.

Citrinin: Among the cereals, maize was predominantly contaminated both with citrinin and AFTB1. Three hundred forty nine samples of maize were positive for citrinin out of which 96 samples also contained AFTB1. Citrinin was present in 251 feed samples and 85% of them were contaminated with AFTB1. Out of the 11 samples of bajra analysed 5 were contaminated with citrinin (100 – 200ppb). Only 6 SBM samples were found to have citrinin and all the samples were negative for AFTB1.

Zearalenone: Out of 79 jowar samples received, 37 jowar samples contained zearalenone ranging from 1 to 2 ppm level. Out of 11 bajra samples, 8 were positive for zearalenone (0.5 to 1ppm).

Emerging new toxins: Apart from the above mentioned toxins the presence of cyclo piazonic acid, oosporin, patulin, deoxynivalenol (DON),

diactoxyscirpernol (DAS), fumonisins and sterigmatocystin have also been recorded occasionally. Further, presence of toxins different from those mentioned above have been suspected due to the fact that mortality has been recorded in birds showing hepatitis and nephritis even though the feed was free from the above mentioned mycotoxins. Several new fluorescent spots are encountered in the analysis of multimycotoxins and some are consistently seen in the feeds obtained from farms with a history of feed related problems, indicating the possibility of new toxins.

The results of the various screening process indicates that the mycotoxin contamination of the feed ingredients has reached an alarming level, where even in dry seasons the feed/ingredients are not free from mycotoxins. The seriousness of the situation has not been well understood either by the agricultural farmers or the raw material suppliers or the poultry farmers.

Field reports of mycotoxicosis in poultry at Namakkal

Based on the test results and interactions with the farmers regarding the effects on the birds, the following observations were made in respect to the various mycotoxins recorded in Namakkal. The need for giving these well known facts again is that these effects were seen even at low levels, whereas, in the literature reports the effects are observed at levels several times above the levels recorded here.

Aflatoxin: Hepatitis, nephritis, immuno suppression. Even at 20 ppb level affected hatchability and even at 40 ppb level caused mortality in chicks.

Citrinin: At 50ppb level caused nephritis in chicks, Immuno suppression, At 40ppb level affected hatchability and at 50ppb level caused watery droppings in layers.

Trichothecenes (T2, DON & DAS): Irritant toxin causing oral ulcers and enteritis, anemia, paleness of beak, legs. Cattle are more sensitive - Feed refusal reported, In an episode (the feed contained 1ppm of T2toxin): the following effects were observed

a) Shriveling of comb b) Moulting a week after exposure c) Regression of ovaries & oviduct.

Ochratoxin: Immuno suppression. At 50ppb level caused nephritis, air sacculitis in chicks and also affected hatchability. At 100ppb level caused watery droppings in layers; affected egg shell thickness and caused leathery eggs.

Oosporin: Nephritis. Gout and Immuno suppression.

Fumonisins: Pasty vent in chicks.

Sterigmatocystin: Hepatitis, at 100ppb level caused nephritis in chicks and watery droppings in growers and layers and Immuno suppression.

Zearalenone: Suspected to cause leathery eggs

The observations made here indicates that in the field conditions the effects of the mycotoxins seen even at such low levels could be due to the presence of two or more mycotoxins and their synergistic effects. Our experience justifies the low permissible levels allowed in some countries.

Mycotoxins in Animal Products: The major concern in mycotoxicosis is not only the decline in health of the livestock and poultry and the consequent economic loss to the farmers, but the residues or the metabolites present in the livestock products affecting the human health. In general nearly 90% of the aflatoxins absorbed are excreted within 24hr. A small amount is retained in the tissues for a longer period of time as they are bound to the proteins, the highest residue concentration was found in the liver. The major concern is the transmission of aflatoxin M1 in the milk because of its carcinogenicity, as the proportion of the toxin in liver to that in the feed was 1:14000 whereas in the milk it was 1:300, which explains the seriousness of the situation. The proportion of the aflatoxin in the feed :broiler liver was 1:1200, swine liver 1: 800 and egg it was 1:2200 (Stoloff, 1980). Similarly the T2 toxin residue in the tissues was also reported (Yoshizawa et al,1981). The tissue: feed ratio in the chicken muscle was 0.014, heart 0.011 and liver 0.027 while in swine muscle it was 0.002, heart 0.003 and liver it was 0.011. The presence of ochratoxin A as high as 29mg in the muscles of chicken collected from a slaughter house was reported by Elling et al., (1975). Kidneys were found to contain the highest levels followed by liver and muscles (Krogh, 1987). Eggs were free of ochratoxins at low levels but were detected

when the feed contained 10mg/kg. Even though the reports on the rate of transfer of mycotoxins to products from feed is very low except in case of M1 to milk, the report of FAO 2002 indicates that contamination of livestock products at field level is possible. This should be taken into consideration while fixing maximum permissible levels of various mycotoxins in the livestock feed and ingredients

The permissible level for various commodities for AFTB1 in most countries is 50ppb or below. In general the EU maximum permissible levels in the feed for various classes of livestock are very low and it is alleged to be used as a discriminatory trade practice. In dairy cattle a level of even 100pbb will not affect the performance of the cow but the major concern is the transmission of aflatoxin M1 through milk and because of its carcinogenicity. As the proportion of toxin accumulated in liver to that in the feed is 1:14000 whereas in the milk it is 1:300, and latest reports claim it is 1:100, the MPL for aflatoxin M1 in milk is 0.5ppb. Based on this a level of 50ppb in the feed will be a justifiable guideline. For poultry the advisable level of mycotoxins in feed is 20ppb and for duck it is 5 ppb, as ducks are very susceptible to mycotoxins.

Current Needs

1. Standardizing a procedure for estimating the currently prevalent mycotoxins in a single run, as the most important thing is that how fast the results can be passed on to the farmer.

2. Simple and economical methods for detoxifying the mycotoxins in feed, as rejecting the feed/ingredients is practically impossible for developing countries like India.

3. Fixing the maximum permissible level of mycotoxins in feed ingredients and feed.

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EU Maximum permitted levels of mycotoxins in animal feed and foods for human consumption

Total aflatoxins	4 ppb dried fruit and nuts
Aflatoxin B1	2 ppb dried fruit and nuts 5 ppb animal feedstuffs – cattle, sheep 2 ppb animal feeding stuffs – adult poultry and swine 1 ppb animal feeding stuffs – piglets and chicks
Ochratoxin A	5 ppb dried fruit and nuts

FDA Guidelines on maximum levels of Aflatoxin, Vomitoxin and Fumonisin in feedstuffs for animals

Aflatoxin	20 ppb dairy, immature pigs and poultry 100 ppb breeding animals 200 ppb finishing swine 300 ppb beef cattle
Vomitoxin	5 ppm swine 10 ppm cattle, poultry
Fumonisins	5 ppm horses 10 ppm swine 50 ppm beef cattle and poultry

Samples screened		Negative	1--20	21 - 50	51 - 100	101 - 200	201 - 500	500 & above	TotaL
		68	722	1388	1671	2091	1555	387	7882
%		0.9	9.2	17.6	21.2	26.5	19.7	4.9	100
Season Total	NR	19	192	492	681	747	596	143	2870
	R	49	530	896	990	1344	959	244	5012
Season %	NR	27.9	26.6	35.4	40.8	35.7	38.3	37.0	36.4
	R	72.1	73.4	64.6	59.2	64.3	61.7	63.0	63.6
% of total samples	Nr	0.7	6.7	17.1	23.7	26.0	20.8	5.0	100
	R	1.0	10.6	17.9	19.8	26.8	19.1	4.9	100

Table 1: Details of Aflatoxin contamination in DOGNC (Concentration in ppb)

Samples screened		Negative	1--20	21 - 50	51 - 100	101 - 200	201 - 500	500 & above	TotaL
		995	3622	692	282	83	39	4	5717
%		17.40	63.35	12.10	4.93	1.45	0.68	0.07	
Season Total	NR	671	1465	67	21	10	2	0	2236
	R	324	2157	625	261	73	37	4	3481
Season %	NR	67.437	40.45	9.682	7.45	12	5.128	0	39.11
	R	32.563	59.55	90.32	92.6	88	94.87	0	60.89
% of total samples	NR	30.01	65.52	3.00	0.94	0.45	0.09	0.00	100.00
	R	9.31	61.96	17.95	7.50	2.10	1.06	0.11	100.00

Table 2: Details of Aflatoxin contamination in SFOC (Concentration in ppb)

Samples screened		Negative	1--20	21 - 50	51 - 100	101 - 200	201 - 500	500 & above	TotaL
		2187	876	299	189	189	144	41	3925
%		55.7	22.3	7.6	4.8	4.8	3.7	1.0	100
Season Total	NR	990	304	72	55	38	62	13	1534
	R	1197	572	227	134	151	82	28	2391
Season %	NR	45.3	34.7	24.1	29.1	20.1	43.1	31.7	39.1
	R	54.7	65.3	75.9	70.9	79.9	56.9	68.3	60.9
% of total samples	NR	64.5	19.8	4.7	3.6	2.5	4.0	0.8	100
	R	50.1	23.9	9.5	5.6	6.3	3.4	1.2	100

Table 3: Details of Aflatoxin contamination in Maize (Concentration in ppb)

Samples screened		Negative	1--20	21 - 50	51 - 100	101 - 200	201 - 500	500 & above	TotaL
		1923	4279	1339	386	263	161	36	8387
%		22.9	51	16	4.6	3.1	1.9	.4	100
Season Total	NR	889	1422	424	112	111	64	27	3049
	R	1034	2857	915	274	152	97	9	5338
Season %	NR	46.23	33.23	31.67	29	42.2	39.75	75	36.35
	R	53.77	66.77	68.33	71	57.8	60.25	25	63.65
% of total samples	NR	29.16	46.64	13.91	3.67	3.64	2.10	0.89	100.00
	R	19.37	53.52	17.14	5.13	2.85	1.82	0.17	100.00

Table 4: Details of Aflatoxin contamination in Poultry feed (Concentration in ppb)

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