Assessment on the incidence of aflatoxin B_1 in composite cattle feed and raw materials in India

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ABSTRACT

*T*Composite cattle feed samples and raw materials like maize, wheat, sorghum, broken rice, pearl millet, groundnut cake, cotton seed cake, mustard cake, soyabean meal, rapeseed meal, sunflower meal, guar meal, safflower meal, deoiled rice bran and wheat bran were collected from various parts of India for a period 5 years and evaluated for aflatoxin B_1 level by screening through ELISA and confirmation through HPLC. Among the 482 composite cattle feed samples, only 68.5per cent were having aflatoxin B_1level below the permissible level (below 20 ppb). Higher incidence of aflatoxin B_1 was recorded in west zone than east, north and south zones during monsoon period. However, it was observed that incidence of aflatoxin B_1 at concentration beyond 100ppb was relatively higher in eastern zone both during monsoon and non-monsoon period than the west, north and south zones. Among the energy source, maize had the highest (7.8 per cent, 15 out of 193 samples tested) prevalence of alarming levels of aflatoxin (>100ppb) and 41.5 per cent (80 samples) in the range of 21 to 100 ppb of aflatoxin B_1. Only 50.8 per cent (98 samples) were within permissible level (<20 ppb). It was observed that the presence of aflatoxin $B_1$was more in maize during monsoon in south zone. Only 47.2 per cent of groundnut cake (118 samples) were within permissible level of <20 ppb of aflatoxin B_1. In west zone, highest sample profile (15.18per cent) and zone-wise distribution of 45.45per cent was recorded in the samples of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100 ppb. Overall data reveals that monsoon favours development of aflatoxin B_1 more during monsoon.

Key Words: Cattle feed, Cakes, Meals, raw materials, Aflatoxin B_1, ELISA, HPLC.

INTRODUCTION

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus*on variety of food products.

Among18 different types of aflatoxins identified, Aflatoxin B_1 is found in the highest concentration and most potent toxin. Aflatoxins are found in many countries, especially in tropical and subtropical regions where conditions of temperature and humidity are optimum for growth of the molds and for production of the toxin. Eliminating them totally from feed and feed ingredients is not an easy task (Azarakhsh *et*

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al., 2011). Aflatoxin B_1 is naturally present in several important feedstuffs like peanut, maize, cotton seed and damaged grains. (Khan *et al.*, 2011). These ingredients are widely used as raw materials in formulation of balanced cattle feed. An important feature in dairying is the fact that aflatoxin B_1 shows a significant carry-over into the milk in its hydroxylated metabolized form of aflatoxin M_1.In view of this, the study was undertaken to generate baseline information on the levels of aflatoxin B_1 in compound cattle feed and raw materials for all over India from different regions at different seasons with the objective to guide compound feed manufacturers to formulate feed based on the intensity of aflatoxins in various feed ingredients, regional prevalence, incidence of occurrence during different season and revise feed formulation accordingly.

MATERIALS AND METHODS

In order to identify regional occurrence of aflatoxin, India was demarcated in this study into four zones viz. north (726133 km^2), south (636236km^2), east (425432km^2) and west (508042 km^2). A total of 2676 samples comprising of commercially available composite cattle feed samples and raw materials like maize (*Zea maize*), sorghum (*Sorghum vulgaris*) mechanical pressed groundnut oil cake (*Arachis hypogeia*), cotton seed oil cake (*Gossypium arboreum*), mustard oil cake (*Brassicanigra)*, soyabean meal (*Glycine max*), rapeseed meal (*Brassica napus)*, solvent extracted cakes of groundnut (*Arachis hypogeia*) andcotton seed (*Gossypium arboreum*), rice bran (*Avena sativa*), deoiledrice bran(*Avena sativa*) and wheat bran (*Triticumaestivum)* were collected from four zones of India during a period of five years. All the samples were collected from different cooperative cattle feed plants and farmers from four zones of India. The east zone contains the states of Odisha, West Bengal, Bihar, Jharkhand, Chattisgarh, Assam, Arunachal Pradesh, Nagaland, Tripura, Mizoram, and Meghalya. The geographical area of east zone is 425432 KM sq.The west zone includes Gujarat, Maharastra, Daman and Diu, Dadra and Nagar Haveli with geographical area to the extent of 508042 KM sq. The North zone includes the states of Jammu Kashmir, Delhi, Punjab, Haryana, Rajasthan, Chandigarh, Himachal Pradesh, Utterpradesh, Uttharkhand and Madhyapradesh with geographical area to the extent of 726133 KM Sq. The south zone includes the states of Tamil Nadu, Andhra Pradesh, Telangana, Karnataka, Kerala, Andaman and Nicobar and Lakshadeep with geographical area to the extent of 636236 KM sq. The samples were collected from above regions throughout the year for the analysis of aflatoxins.

In order to identify seasonal variation, the periods were separated into two, namely non-monsoon and monsoon seasons according to local meteorological parameters. The samples were finely ground in a willy mill and one kg material was mixed thoroughly for homogeneity. Portion of 100g sub samples were drawn for aflatoxin analysis by HPLC and ELISA methods. The samples were screened by ELISA method for aflatoxin B_1 (Patey *et al.,*1992). Acetonitrile was used to extract toxins from the ground-up sample. In ELISA method, the limited level (Max. 0.4 ppb) of aflatoxin B_1could be tested.

The samples that were beyond the deduction level of ELISA method (50ppb) were analysed by HPLC. The feed sample was extracted and purified as per AOAC (2012).The HPLC conditions were used to separate the aflatoxin B_1. Mobile phase: Actonitrile: Water (65: 35) with 0.5ml flow rate, ambient temperature for column (C18 ODS, 125 x 4mm), Florescence detector: Ex. 365nm and Em. 430nm. The sensitivity for aflatoxin detection by HPLC was 20picogram.The samples were quantified in the ranges of 0-20ppb (IS 2052:2009) recommended level) and considered as "safe", while aflatoxin B_1 from 21 to 100ppb was considered as "High". Since production and health of dairy herds are affected at dietary aflatoxin levels above 100ppb which is considerably higher than the amount that produces illegal milk residues (Patterson and Anderson 1982 and Masri *et al.*, 1969), concentration of aflatoxin B_1 more than 100ppb was considered as 'Alarmingly high'.

RESULTS AND DISCUSSIONS

Average aflatoxin B_1 (ppb) content in the composite cattle feed and raw materials are presented in Table 1. The average level of aflatoxin B_1 with Mean± SE in 482 samples of composite cattle feed tested across India was 46.40 ± 2.68 with 68.5per cent of samples within permissible level(<20 ppb) and 2.3per cent(11 numbers) at alarming high (>100ppb) level. Several authors have reported aflatoxin B_1 levels varying from 100 to 1000ppb. (Reddy *et al.,* 2000; Lanyasunya *et al*., 2005; Charoenpornsook and Kavisarasai, 2006; Martins *et al.,* 2007). Similar incidence of contamination of alflatoxin B_1 (33per cent) in composite feed in India was recorded Kotinagu *et al.* (2015). The global prevalence of aflatoxin positive samples and the mean concentration were reported to be 33per cent and 21 ppb, respectively (Andrea *et al*., 2017). Incidence of multiple aflatoxicosis outbreaks have been reported worldwide, in Kenya (Kangíethe and Langía, 2009), in Thailand (Charoenpornsook and Kavisarasai., 2006).Countries in Europe including Romania, Serbia, and Croatia also reported the aflatoxin contamination of milk nationwide, Abbas *et al.* (2010). The carry-over level of aflatoxin B_1 to aflatoxin M_1 varies from 0.1per cent to 5per cent. (Lynch, 1971; Fremy *et al.,* 1988; Hans *et al.,* 2000; Garg *et al.,* 2004).A thumb rule is that milk aflatoxin concentrations replace equal about 1.7per cent of the aflatoxin concentration in the total ration dry matter (Whitlow and Hagler, 2005). Thus, cows consuming diets containing 46 ppb aflatoxin will produce milk containing aflatoxin residues of about 0.78 ppb which is 15 times higher than 0.05 ppb which is the safe limit for aflatoxin M_1 in milk (Codex, 2001).Five energy source, eight protein supplements and three by products that are commonly included as feed ingredients in composite cattle feed were also tested. Among energy source, maize had the highest (7.8per cent of 193 samples tested) prevalence of alarming level (>100ppb)and 41.5per cent in the range of 21 to 100 ppb of aflatoxin B_1. Only 50.8per cent(98 samples)were within permissible level (<20ppb). The mean ± SE level of aflatoxin B_1 in 193 samples of maize was found to be 61.75 ± 16.95 and was the second most highly significant ($P<0.01$) infested feed ingredient that is widely used in cattle feed. However, the level of aflatoxin B_1 in maize was highly variable as reflected

by high standard error associated to mean value. Next in the order of prevalence of aflatoxin B_1 among energy source was broken rice. Though 80.4per cent of broken rice (194 samples) had aflatoxin B_1 within permissible level, the mean ± SE of 36.60 ± 8.59 was significantly (P<0.01) higher than the rest of energy sources tested. Further broken rice had alarming level of aflatoxin B_1 in 2.0per cent of samples tested. The wheat, sorghum and Pearl millets with 85.8, 96.0and 100per cent of 155, 125 and 121 respective samples tested were relatively safe with aflatoxin B_1 level less than 20ppb. Higher incidence of aflatoxin B_1 in maize at higher concentration recorded in this study concurs with the results (1000ppb) published by Sinha (1987).

Table 1. Prevalence of aflatoxin B_1 in composit cattle feed and feed ingredients in India

Sr. No	Ingredients	No. of Samples	Aflatoxin B_1 (ppb) mean with SE*	Aflatoxin B_1 Concentraztion (ppb) and its percentage of Samples tested					
				ND-20	% Safe	21-100	% High	>100	% Alarming
A	**Composite Feed**								
1	Composite Cattle feed	482	46.40 ± 2.68^{c}	330	68.5	141	29.3	11	2.3
B	**Energy source**								
1	Maize	193	61.75 ± 16.95^{b}	98	50.8	80	41.5	15	7.8
2	Wheat	155	28.43 ± 9.85^{e}	133	85.8	22	14.2		0.0
3	Sorghum	125	8.66 ± 0.67^{g}	120	96.0	5	4.0		0.0
4	Broken rice	194	36.60 ± 8.59^{d}	156	80.4	36	18.6	2	1.0
5	Pearl millet	121	9.55 ± 1.45^{g}	121	100.0	0	0.0		0.0
C	**Protein supplement**								
1	Groundnut Cake	250	71.16 ± 9.17^{a}	118	47.2	110	44.0	22	8.8
2	Cotton Seed Cake	151	46.29 ± 7.66^{c}	128	84.8	23	15.2		0
3	Mustard cake	145	13.42 ± 9.68^{fg}	125	86.2	20	13.8		0
4	Rape seed meal	134	17.44 ± 2.55^{f}	116	86.6	18	13.4		0
5	Soyabean meal	152	11.19 ± 7.01^{g}	134	88.2	18	11.8		0
6	Sunflower meal	132	16.01 ± 9.76^{f}	124	93.9	8	6.1		0
7	Guar meal	117	8.43 ± 3.16^{g}	117	100.0	0	0.0		0
8	Safflower meal	112	9.57 ± 8.89^{g}	112	100.0	0	0.0		0
D	**By Products**								
1	Deoiled rice bran	81	10.70 ± 6.91^{g}	77	95.1	4	4.9		0
2	Wheat bran	132	12.71 ± 9.35^{fg}	128	97.0	4	3.0		0

*Means bearing different alphabets in a column (p<0.01) highly Significantly

The highest (8.8per cent of 250 samples) incidence of alarming level (>100ppb) aflatoxin $B_1$was recorded in groundnut cake. Only 47.2per cent of Groundnut cake (118 samples) were within permissible level of <20ppb of aflatoxin B_1. The mean level of aflatoxin B_1 with SE (71.16 ± 9.17) was significantly (P<0.01) highest in Groundnut cake across all samples tested. Similar observation of 41 to 51per cent of maize samples being infested in Kenya was reported by Johnni *et al.* (2011). Sharma *et al.* (1994) reported highest level of 2000ppb in groundnut cake and Bhat *et al.* (1996) reported maximum level of contamination of 833ppb in the state of Gujarat.

The cotton seedcake was the next highest source of aflatoxin B_1among protein supplements with 84.8per cent of 151 samples tested having <20ppb and mean level of aflatoxin B_1 with SE of 46.29 ± 7.66. The mean ± SE of cotton seed cake was significantly (P<0.01) higher than the rest of protein supplements tested. Other protein supplements like mustard cake, rape seed meal, soyabean meal, sunflower meal, gaur meal and safflower meal were relatively safe with aflatoxin B_1 at less than 20ppb in 86.2, 86.6, 88.2, 93.0, 100 and 100per cent respectively of 145, 134, 152, 132, 117 and 112 samples tested.

None of the by-products namely, deoiled rice bran and wheat bran had aflatoxin B_1 at alarming level and majority (95.1, 97.0per cent of 81 and 132respectively of the samples tested) were safe with aflatoxin B_1 within permissible level. The mean and SE values were significantly (P<0.01) lower with10.70 ± 6.91 and 12.71 ± 9.35, respectively.

Phitsanu and Yoshiko (2017) observed that 30.5per cent of peanuts, corn and rice showed an aflatoxin B_1 contamination ranging from 0.01-626ppb that are very similar to the observations made in this study. Maize, groundnuts and rice represent the most common sources of food supply contamination worldwide, as these crops are preferred by *Aspergillus* for colonization pre-harvest, while also being susceptible to contamination due to improper drying and storage conditions post-harvest (CAST, 2003).

The incidence of aflatoxin B_1 in composite cattle feed and raw materials tested from four zones in India are presented in table 2. The table classifies data as "sample profile" which denotes the proportion of aflatoxins B_1 concentration within each zone. This data was reclassified to represent zone wise distribution. In order to elicit the effect of environment, the data was divided according to period of origin (monsoon and non-monsoon) and presented zone-wise as well as across zones to reveal overall picture in Table 3.Thecomposite cattle feed in west zone recorded lowest sample profile (60per cent) and zone-wise distribution percentage (21.69) of samples within the permissible level (<20ppb) of aflatoxin B_1,while in other three zones, sample profile ranged from 68.46 to 72.81 and zone-wise distribution ranged from 25.00 to 26,81per cent. This is indicative of higher incidence of aflatoxin B_1 in west zone than other three zones. Data rearranged across zone to elicit the effect of monsoon indicate that relatively lower proportion (67.86per cent) of composite cattle feed were within safety limit (<20ppb) during monsoon compared to 74.42per cent in non-monsoon period. Period-wise assessment in each zone on the percentage of samples falling within safety limit revealed that only 31.43per cent of the composite cattle feed samples analysed were within safety limit in west zone during monsoon, while it ranged from 56.25 to 67.86 in other zones. During non-monsoon period, these values ranged from 71.46 to 75.00 per cent in four zones. Overall data reveals that monsoon favours the development of aflatoxin B_1 more during monsoon in west zone than other zones. This observation is further reinforced by the data pertaining to incidence of aflatoxin B_1concentrations between 21 to 100ppb wherein the

percentage of samples at west zone during monsoon was 65.71 as against the range of 28.13to 39.29 in other three zones. In contrast, it was observed that incidence of aflatoxin B_1 at concentration beyond 100ppb was relatively higher in eastern zone both during monsoon and non-monsoon period than the west, north and south zone. Thus, seasonal changes in ingredient composition of composite cattle feed or conducive environment during monsoon alone cannot be attributed to incidence of aflatoxin B_1 as no uniform pattern were noticed. However, contaminations with *Aspergillus flavus* as well as the presence of aflatoxin B_1 have been linked with the onset and intensity of the monsoon and subsequent storage conditions of the feed (Mehan and McDonald, 1983).The lower incidence of aflatoxin B_1 at concentration below100ppb and higher incidence at beyond 100ppb in east zone indicates that at some pockets the composite cattle feed were stored for longer duration leading to skewing up higher incidence at beyond 100ppb.In concurrence to the observation made in this study, higher incidence of aflatoxin B_1upto 833ppb was observed in the state of Gujarat (Bhat *et al.,* 1996), which falls under western zone.

Table 2. Incidence of aflatoxin B_1 (Mean and respective percentage of total) in composite cattle feed and raw materials tested from four Zones in India.

Sr. No	Ingredients	Level of aflatoxin B1 (ppb)	No of samples / percentage from different sources at four zones												
			Sample profile								Zone wise percetage of sample				
			East		West		North		South						
	Seasons		Number of sample	Profile Percentage	Number of sample	Profile Percentage	Number of sample	Profile Percentage	Number of sample	Profile Percentage	East	West	North	South	Total
A	Composite cattle feed	ND -20	89	68.46	72	60.00	83	72.81	88	72.13	26.81	21.69	25.00	26.51	100.00
		21-100	32	24.62	46	38.33	31	27.19	34	27.87	22.38	32.17	21.68	23.78	100.00
		>100	9	6.92	2	1.67	0	0.00	0	0.00	81.82	18.18	0.00	0.00	100.00
		Total	**130**	**100.00**	**120**	**100.00**	**114**	**100.00**	**122**	**100.00**					
B	Grains														
1	Maize	ND -20	25	48.08	26	52.00	26	46.43	21	38.89	25.51	26.53	26.53	21.43	100.00
		21-100	23	44.23	21	42.00	27	48.21	28	51.85	23.23	21.21	27.27	28.28	100.00
		>100	4	7.69	3	6.00	3	5.36	5	9.26	26.67	20.00	20.00	33.33	100.00
		Total	**52**	**100.00**	**50**	**100.00**	**56**	**100.00**	**54**	**100.00**	**75.41**	**67.74**	**73.80**	**83.04**	
2	Wheat	ND -20	25	78.13	34	89.47	21	91.30	18	66.67	25.51	34.69	21.43	18.37	100.00
		21-100	7	21.88	4	10.53	2	8.70	9	33.33	31.82	18.18	9.09	40.91	100.00
		Total	**32**	**100.00**	**38**	**100.00**	**23**	**100.00**	**27**	**100.00**					
3	Sorghum	ND -20	31	100.00	34	97.14	27	96.43	29	93.55	25.62	28.10	22.31	23.97	100.00
		21-100	0	0.00	1	2.86	1	3.57	2	6.45	0.00	25.00	25.00	50.00	100.00
		Total	**31**	**100.00**	**35**	**100.00**	**28**	**100.00**	**31**	**100.00**					
4	Broken rice	ND -20	37	82.22	49	96.08	37	77.08	33	66.00	23.72	31.41	23.72	21.15	100.00
		21-100	8	17.78	2	3.92	11	22.92	15	30.00	22.22	5.56	30.56	41.67	100.00
		>100	0	0.00	0	0.00	0	0.00	2	4.00	0.00	0.00	0.00	100.00	100.00
		Total	**45**	**100.00**	**51**	**100.00**	**48**	**100.00**	**50**	**100.00**					
3	Pearl millet	ND -20	31	100.00	31	100.00	22	100.00	37	100.00	25.62	25.62	18.18	30.58	100.00
		Total	**31**	**100.00**	**31**	**100.00**	**22**	**100.00**	**37**	**100.00**					
C	Protein meals/Oil Cakes														
1	Groundnut cake	ND -20	28	46.67	25	38.46	28	45.90	37	57.81	23.73	21.19	23.73	31.36	100.00
		21-100	27	45.00	30	46.15	28	45.90	25	39.06	24.55	27.27	25.45	22.73	100.00
		>100	5	8.33	10	15.38	5	8.20	2	3.13	22.73	45.45	22.73	9.09	100.00
		Total	**60**	**100.00**	**65**	**100.00**	**61**	**100.00**	**64**	**100.00**					
2	Cottonseed cake	ND -20	22	61.11	26	63.41	22	61.11	24	63.16	23.40	27.66	23.40	25.53	100.00
		21-100	14	38.89	15	36.59	14	38.89	14	36.84	24.56	26.32	24.56	24.56	100.00
		Total	**36**	**100**	**41**	**100**	**36**	**100**	**38**	**100**					

3	Mustard cake	ND -20	36	85.71	35	85.37	34	87.18	20	86.96	28.80	28.00	27.20	16.00	100.00
		21-100	6	14.29	6	14.63	5	12.82	3	13.04	30.00	30.00	25.00	15.00	100.00
		Total	**42**	**100**	**41**	**100**	**39**	**100**	**23**	**100**					
4	Rape seed meal	ND -20	32	86.49	34	87.18	31	86.11	19	86.36	27.59	29.31	26.72	16.38	100.00
		21-100	5	13.51	5	12.82	5	13.89	3	13.64	27.78	27.78	27.78	16.67	100.00
		Total	**37**	**100**	**39**	**100**	**36**	**100**	**22**	**100**					
5	Soyabean meal (extracted)	ND -20	41	89.13	30	85.71	35	89.74	28	87.50	30.60	22.39	26.12	20.90	100.00
		21-100	5	10.87	5	14.29	4	10.26	4	12.50	27.78	27.78	22.22	22.22	100.00
		Total	**46**	**100**	**35**	**100**	**39**	**100**	**32**	**100**					
6	Sunflower meal	ND -20	28	93.33	31	93.94	31	93.94	34	94.44	22.58	25.00	25.00	27.42	100.00
		21-100	2	6.67	2	6.06	2	6.06	2	5.56	25.00	25.00	25.00	25.00	100.00
		Total	**30**	**100**	**33**	**100**	**33**	**100**	**36**	**100.00**					
7	Guar meal	ND -20	29	100	31	100.00	31	100.00	26	100.00	24.79	26.50	26.50	22.22	100.00
		Total	**29**	**100**	**31**	**100**	**31**	**100**	**26**	**100.00**					
8	Safflower meal	ND -20	28	100	27	100.00	29	100.00	28	100.00	25.00	24.11	25.89	25.00	100.00
		Total	**28**	**100**	**27**	**100**	**29**	**100**	**28**	**100.00**					
D	**Brans**														
1	Deoiled Rice bran	ND -20	17	94.44	19	95.00	18	94.74	23	95.83	22.08	24.68	23.38	29.87	100.00
		20-100	1	5.56	1	5.00	1	5.26	1	4.17	25.00	25.00	25.00	25.00	
		Total	**18**	**100**	**20**	**100**	**19**	**100**	**24**	**100.00**					
2	Wheat bran	ND -20	35	97.22	36	97.30	28	96.55	29	96.67	27.34	28.13	21.88	22.66	100.00
		20-50	1	2.78	1	2.70	1	3.45	1	3.33	25.00	25.00	25.00	25.00	
		Total	**36**	**100**	**37**	**100**	**29**	**100**	**30**	**100.00**					

The maize sample profile data of south zone suggest that prevalence of aflatoxin B_1 below 20ppb was 38.89per cent of samples received and was relatively lower than other three zones. Zone wise segregation of samples also revealed only 21.43per cent of samples from south zone were within safe limit of aflatoxin B_1. Incidentally, maize is not largely consumed by human in south zone. Maize is widely cultivated and consumed by human in west and north zones. Period-wise occurrence of aflatoxin B_1 in maize at concentration above 20ppb across four zones indicated that samples tested during monsoon were more prone to aflatoxin B_1 than those tested during non-monsoon period even though non-monsoon lasts for ten months as against two months of monsoon period. Similarly, the sample profile, zone-wise distribution of samples, period-wise incidence in each zone and across all zones of other cereals like wheat, sorghum and broken rice were all found to be higher during monsoon in south zone than other three zones. All the pearl millet samples analysed were within safe limit of aflatoxin B_1. Thus, it is evident that prevalence of aflatoxin B_1 in cereals was found to be higher during monsoon in south zone than other three zones.

The incidence of aflatoxin B_1 in groundnut cake was found to be highest among all samples tested. In contrast to cereals, south zone samples of groundnut cake were found to have lower incidence of aflatoxin B_1 as indicated by sample profile data and zone-wise segregation of sample results. The west zone recorded lowest sample profile (38.46per cent) and the zone profile (21.19per cent) of samples within the permissible level of (<20ppb) aflatoxin B_1, while in other three zones sample profile ranged from 45.90per cent to 57.81per cent and zone-wise distribution ranged from 23.73 to 31.36per cent. In west

Table 3. Incidence of aflatoxin B_1 (Mean and respective percentage of total) in composite cattle feed and raw materials tested at two seasons from four Zones in India.

Sr. No	Ingredients	Level of aflatoxin B1 (ppb)	No of samples / percentage from different sources at four zones in different periods																			
			Sample profile - Period wise in each zone																Period wise across zone			
			East				West				North				South							
	Seasons		Non monsoon	(% during non monsoon within zone)	Monsoon	(% during non monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)
A	Composite cattle feed	ND -20	71	74.0	18	56.3	61	71.8	11	31.4	64	74.4	19	67.9	69	75.0	17	60.7	265	73.8	65	52.8
		21-100	21	21.9	9	28.1	23	27.1	23	65.7	22	25.6	9	32.1	23	25.0	11	39.3	89	24.8	52	42.3
		>100	4	4.2	5	15.6	1	1.2	1	2.9	0	0.0	0	0.0	0	0.0	0	0.0	5	1.4	6	4.9
		Total	**96**	**100.0**	**32**	**100.0**	**85**	**100.0**	**35**	**100.0**	**86**	**100.0**	**28**	**100.0**	**92**	**100.0**	**28**	**100.0**	**359**	**100.0**	**123**	**100.0**
B	**Grains**																					
1	Maize	ND -20	19	51.4	6	40.0	23	59.0	3	27.3	24	77.4	2	33.3	18	51.4	3	15.8	84	59.2	14	27.5
		21-100	16	43.2	7	46.7	15	38.5	6	54.5	5	16.1	3	50.0	15	42.9	13	68.4	51	35.9	29	56.9
		>100	2	5.4	2	13.3	1	2.6	2	18.2	2	6.5	1	16.7	2	5.7	3	15.8	7	4.9	8	15.7
		Total	**37**	**100.0**	**15**	**100.0**	**39**	**100.0**	**11**	**100.0**	**31**	**100.0**	**6**	**100.0**	**35**	**100.0**	**19**	**100.0**	**142**	**100.0**	**51**	**100.0**
2	Wheat	ND -20	15	83.3	20	83.3	48	96.0	11	84.6	10	90.9	11	91.7	15	83.3	3	33.3	88	90.7	45	77.6
		21-100	3	16.7	4	16.7	2	4.0	2	15.4	1	9.1	1	8.3	3	16.7	6	66.7	9	9.3	13	22.4
		Total	**18**	**100.0**	**24**	**100.0**	**50**	**100.0**	**13**	**100.0**	**11**	**100.0**	**12**	**100.0**	**18**	**100.0**	**9**	**100.0**	**97**	**100.0**	**58**	**100.0**
3	Sorghum	ND -20	22	100.0	9	100.0	16	100.0	18	94.7	21	100.0	6	85.7	12	92.3	17	94.4	71	98.6	50	94.3
		21-100	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	1	14.3	1	7.7	1	5.6	1	1.4	3	5.7
		Total	**22**	**100.0**	**9**	**100.0**	**16**	**100.0**	**19**	**100.0**	**21**	**100.0**	**7**	**100.0**	**13**	**100.0**	**18**	**100.0**	**72**	**100.0**	**53**	**100.0**
4	Broken rice	ND -20	23	82.1	14	82.4	31	96.9	18	94.7	24	82.8	13	68.4	21	70.0	12	60.0	99	83.2	57	76.0
		21-100	5	17.9	3	17.6	1	3.1	1	5.3	5	17.2	6	31.6	8	26.7	7	35.0	19	16.0	17	22.7
		>100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3	1	5.0	1	0.8	1	1.3
		Total	**28**	**100.0**	**17**	**100.0**	**32**	**100.0**	**19**	**100.0**	**29**	**100.0**	**19**	**100.0**	**30**	**100.0**	**20**	**100.0**	**119**	**100.0**	**75**	**100.0**
3	Pearl millet	ND -20	15	100.0	16	100.0	11	100.0	20	100.0	**4**	100.0	18	100.0	**14**	100.0	23	100.0	44	100.0	77	100.0
		Total	**15**	**100.0**	**16**	**100.0**	**11**	**100.0**	**20**	**100.0**	**4**	**100.0**	**18**	**100.0**	**14**	**100.0**	**23**	**100.0**	**44**	**100.0**	**77**	**100.0**
C	**Protein meals/Oil Cakes**																					
1	Groundnut cake	ND -20	14	48.3	14	45.2	13	44.8	12	33.3	14	51.9	14	41.2	19	59.4	18	56.3	60	51.3	58	43.6
		21-100	14	48.3	13	41.9	12	41.4	18	50.0	11	40.7	17	50.0	13	40.6	12	37.5	50	42.7	60	45.1
		>100	1	3.4	4	12.9	4	13.8	6	16.7	2	7.4	3	8.8	0	0.0	2	6.3	7	6.0	15	11.3
		Total	**29**	**100.0**	**31**	**100.0**	**29**	**100.0**	**36**	**100.0**	**27**	**100.0**	**34**	**100.0**	**32**	**100.0**	**32**	**100.0**	**117**	**100.0**	**133**	**100.0**

2	Cottonseed cake	ND -20	11	57.9	11	64.7	15	71.4	11	55.0	13	68.4	9	52.9	12	70.6	12	57.1	51	67.1	43	57.3
		21-100	8	42.1	6	35.3	6	28.6	9	45.0	**6**	**31.6**	8	47.1	5	29.4	9	42.9	25	32.9	32	42.7
		Total	**19**	**100.0**	**17**	**100.0**	**21**	**100.0**	**20**	**100.0**	**19**	**100.0**	**17**	**100.0**	**17**	**100.0**	**21**	**100.0**	**76**	**100.0**	**75**	**100.0**
3	Mustard cake	ND -20	18	81.8	18	90.0	14	87.5	21	84.0	22	95.7	12	75.0	4	80.0	16	88.9	58	87.9	67	84.8
		21-100	4	18.2	2	10.0	2	12.5	4	16.0	1	4.3	4	25.0	1	20.0	2	11.1	8	12.1	12	15.2
		Total	**22**	**100.0**	**20**	**100.0**	**16**	**100.0**	**25**	**100.0**	**23**	**100.0**	**16**	**100.0**	**5**	**100.0**	**18**	**100.0**	**66**	**100.0**	**79**	**100.0**
4	Rape seed meal	ND -20	22	91.7	10	76.9	23	92.0	11	78.6	18	90.0	13	81.3	10	100.0	9	75.0	73	92.4	43	78.2
		21-100	2	8.3	3	23.1	2	8.0	3	25.0	2	10.0	3	18.8	0	0.0	3	25.0	6	7.6	12	21.8
		Total	**24**	**100.0**	**13**	**100.0**	**25**	**100.0**	**14**	**103.6**	**20**	**100.0**	**16**	**100.0**	**10**	**100.0**	**12**	**100.0**	**79**	**100.0**	**55**	**100.0**
5	Soyabean meal (extracted)	ND -20	23	92.0	18	85.7	18	90.0	12	80.0	19	90.5	16	88.9	19	90.5	9	81.8	79	90.8	55	84.6
		21-100	2	8.0	3	14.3	2	10.0	3	20.0	2	9.5	2	11.1	2	9.5	**2**	18.2	8	9.2	10	15.4
		Total	**25**	**100.0**	**21**	**100.0**	**20**	**100.0**	**15**	**100.0**	**21**	**100.0**	**18**	**100.0**	**21**	**100.0**	**11**	**100.0**	**87**	**100.0**	**65**	**100.0**
6	Sunflower meal	ND -20	18	100.0	10	83.3	17	100.0	14	87.5	16	94.1	15	93.8	18	100.0	16	88.9	69	98.6	55	88.7
		21-100	0	0.0	2	16.7	0	0.0	2	12.5	1	5.9	1	6.3	0	0.0	2	11.1	1	1.4	7	11.3
		Total	**18**	**100.0**	**12**	**100.0**	**17**	**100.0**	**16**	**100.0**	**17**	**100.0**	**16**	**100.0**	**18**	**100.0**	**18**	**100.0**	**70**	**100.0**	**62**	**100.0**
7	Guar meal	ND -20	14	100.0	15	100.0	13	100.0	18	100.0	15	100.0	16	100.0	0	0.0	0	**0.0**	55	100.0	62	100.0
		Total	**14**	**100.0**	**15**	**100.0**	**13**	**100.0**	**18**	**100.0**	**15**	**100.0**	**16**	**100.0**	**0**	**0.0**	**0**	**0.0**	**55**	**100.0**	**62**	**100.0**
8	Safflower meal	ND -20	**13**	**100.0**	15	100.0	14	100.0	**13**	100.0	14	100.0	**15**	100.0	13	100.0	15	100.0	54	100.0	58	100.0
		Total	**13**	**100.0**	**15**	**100.0**	**14**	**100.0**	**13**	**100.0**	**14**	**100.0**	**15**	**100.0**	**13**	**100.0**	**15**	**100.0**	**54**	**100.0**	**58**	**100.0**
D	**Brans**																					
1	Deoiled Rice bran	ND -20	11	100.0	6	85.7	12	100.0	7	87.5	10	100.0	8	88.9	11	100.0	12	92.3	44	100.0	33	89.2
		20-100	0	0.0	1	14.3	0	0.0	1	12.5	0	0.0	1	11.1	0	0.0	1	7.7	0	0.0	4	10.8
		Total	**11**	**100.0**	**7**	**100.0**	**12**	**100.0**	**8**	**100.0**	**10**	**100.0**	**9**	**100.0**	**11**	**100.0**	**13**	**100.0**	**44**	**100.0**	**37**	**100.0**
2	Wheat bran	ND -20	17	100.0	18	94.7	12	100.0	24	96.0	12	100.0	16	94.1	15	100.0	14	93.3	56	100.0	72	94.7
		20-50	0	0.0	1	5.3	0	0.0	1	4.0	0	0.0	1	5.9	0	0.0	1	6.7	0	0.0	4	5.3
		Total	**17**	**100.0**	**19**	**100.0**	**12**	**100.0**	**25**	**100.0**	**12**	**100.0**	**17**	**100.0**	**15**	**100.0**	**15**	**100.0**	**56**	**100.0**	**76**	**100.0**

zone, highest sample profile (15.18per cent) and zone-wise distribution of 45.45per cent was recorded in the samples of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100 ppb. Though the overall incidence of aflatoxin B_1across zone in groundnut cake was higher during monsoon period, the south zone recorded lower incidence during monsoon than non-monsoon period.

Data on cottonseed cake sample profile and zone-wise segregation of samples revealed that prevalence of aflatoxin B_1 was uniformly infested across zone and incidence was higher during monsoon. Though mustard seed sample profile also indicated uniform distribution within respective zone, zone-wise segregation of samples revealed that prevalence of aflatoxin B_1 was relatively more in east and west zone with south being the least. Similarly, Rapeseed meal also revealed uniform sample profile with least prevalence of aflatoxin B_1 in south zone. Soyabean meal and sunflower meal were uniformly infested both on sample profile as well as on zone-wise assessment similar to cotton seed cake. All the samples of guar meal and safflower meal tested were within the safe limit of aflatoxin B_1.The incidence of aflatoxin B_1 was higher during monsoon in mustard seed, rapeseed meal, soyabean meal and sunflower meal. The overall prevalence of aflatoxin B_1 was least in protein supplements and highest in cereals in south zone.

Samples of deoiled rice bran and wheat bran revealed uniform aflatoxin B_1 profile as well as similar zone-wise distribution and the incidence of aflatoxin B_1 occurred only during monsoon.

CONCLUSIONS

This study was conducted to generate the baseline information on the level of aflatoxin B_1 content in compound cattle feed and all raw materials from different regions of country. Aflatoxin B_1 was found to be at an alarming level in most commonly used feed ingredients (> 100 ppm: groundnut cake 8.8per cent, maize7.8per cent) as well in composite cattle feed (2.3per cent) among 2676samples tested. Only 68.5 per cent of 482 composite cattle feed samples analysed were safe with aflatoxin B_1 below 20ppb. Higher incidence of aflatoxin B_1 was recorded in west zone than east, north and south zones during monsoon period. Thus, seasonal changes in ingredient composition of composite cattle feed or conducive environment during monsoon alone cannot be attributed to incidence of aflatoxin B_1 as no uniform pattern were noticed. Among energy source, maize had the highest prevalence of alarming level (>100ppb) and it was observed that the incidence of aflatoxin $B_1$was more in maize during monsoon in south zone. In west zone, highest sample profile (15.18per cent) of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100ppb.Overall data reveals that monsoon favours development of aflatoxin $B_1$is more during monsoon. This study reveals that no single factor can be attributed to the high incidence of aflatoxin B1, though the major ingredients viz., maize and groundnut cake were found to be highly prone to aflatoxin B_1. However, it is strongly recommended not to source maize from south zone during monsoon period and groundnut from west zone during monsoon period. During monsoon period, ration formulation with

sorghum and peral millet as energy source and sunflower meal, guar meal, safflower meal for protein source can be considered in addition to by products like deoiledrice bran and wheat bran. This study recommends stringent action to control aflatoxin B_1 to ensure not only animal health but also to prevent its cascading effect on human health.

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