Detection of Aflatoxin B1 (AFB1) in the common ingredients of poultry and broiler feed under different seasons

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Abstract

A survey work was conducted to detect the level of AFB1 in the poultry feed *viz*. pre-broiler starter, broiler starter and broiler finisher and selected feed ingredients like maize, de-oiled rice bran, groundnut oil cake, soybean meal, broken rice and de-oiled sunflower cake that were received and analyzed during the period of one year from 17th october 2019 to 16th october 2020 at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal. Among the samples, highest level of contamination was noticed in groundnut oil cake, maize and de-oiled rice bran at a level more than 100 µg / kg followed by broiler pre starter, broiler starter feed, broiler finisher feed, broken rice and soybean meal during the northeast monsoon, non-monsoon and southwest monsoon period with more number of samples received during southwest monsoon. Hence surveillance is essential at every stage to identify mycotoxins starting from raw material production to production of finished feed storage and before use in the livestock and poultry feed.

Key words: Aflatoxin B1; Poultry Feed; Feed Ingredients; Season; Permissible level

Introduction

Mycotoxicosis produced by mycotoxins remained as the "neglected disease" in both humans and animals until the early sixties. As time advance, there was a greater impact imposed by these toxins on both human and animal population which changed the attitude and lead to the implementation of strict regulatory measures. It became very clear that the presence of these mycotoxins in foods and feeds are undesirable and should be avoided or otherwise kept as low as possible. Today the laws governing the food industry not only prohibit the introduction of these toxins but also include specific regulations that impose certain limits or level of acceptance on the concentration of particular contaminants in foods, which may either be of industrial or natural origin. Since mycotoxins are natural contaminants, certain level of toxins are unavoidable and their exposure must be tolerated (Pittet, 1998). Among the various types of toxins produced, aflatoxin (AF) was first noted in early 1960's and caused significant health concern since then with persistent problems in food trading. To overcome these issues, certain regulations specific to mycotoxin contamination were framed, developed and followed in several countries, initially referring only to AF but later regulations governing the safety limit for other mycotoxins such as deoxynivalenol, Ochratoxin, patulin, citrinin and zearalenone were also included in the food for some countries.

The choice of fixing limits for mycotoxin contamination depends on the availability of toxicological data, data on the prevalence of contamination in various food commodities and feed materials, availability of the different methods of sampling, analysis of toxins in the commodities, implications for intercountry trade and the existence of sufficient food supply.

From a regulatory standpoint, *AF* are considered to be unavoidable contaminants in food since they cannot be completely prevented or eliminated by the existing good agricultural practices. Thus, exposure to some level of mycotoxins is tolerated by the population. However, setting up the maximum levels of *AF* in foods and feeds is generally essential and recognized. Several countries, particularly most of the industrialized nations, have already set the limits, ranging from 0 to 30 µg / kg for aflatoxin B1 (*AF*B1) in foodstuffs and from 0 to 50 µg / kg for total *AF* contamination. The setting of limits which are internationally agreed for the maximum tolerable levels for *AF* in food and feed is of global importance. The Joint FAO / WHO Expert Committee on Food Additives (JECFA) concluded that there is no significant difference in risk to human health between the maximum levels of 10 µg / kg and 20 µg / kg for AFB1 in food (*Boutrif, 1997).* The Food and Drug Administration (FDA) in the United States has given regulation for the allowable AF contamination as 20 ppb in crops and 0.5 ppb in milk for humans. In Europe, foods containing more than 2 ppb of AFB1 or 4 ppb of AF were strictly rejected, whereas in India, the limit of AF contamination is 30 ppb in food commodities (Van Egmond and Jonker, 2004).

Thus the limits of AFB1, AFB2, AFG1, AFG2 and AFM1 (0 - 40 ppb for foods and 0 – 1000 ppb for feed); for OTA (0-50 ppb in food and 0-1000 ppb in feed); for Don (500 – 2000 ppb in food and 5 - 10,000 ppb in feed); for zearalenone (0 - 1000 ppb in food); for patulin (0 - 50 ppb in foods), for diacetoxyscirpenol (0 - 100 ppb in feed); for chetomin (0 ppb in feed); for stachybotryotoxin (0 ppb in feeds) and for fumonisins (0 - 1000 ppb in food, 5000-50,000 ppb in feedstuffs). Thus, the set limits promote a global harmonization for mycotoxin regulations and control procedures and facilitate international food trade. These regulations are followed based on sound scientific principles and risk analysis which in turn helps to make recommendation on strategies for further preventing and controlling mycotoxin contamination with minimized food losses and therby ensuring the safety and wholesomeness of food and feed supply (Mazumder and Sasmal, 2001).

Hence, with this background, a survey work was carried out to screen the poultry feed and feed ingredients for the most toxic aflatoxin B1 (AFB1) contamination for a period of one year to find out the feed and feed ingredient most prone for contamination and to find out the seasonal influence.

Materials and Methods

AFB1 was detected in the routine poultry feeds *viz*. pre-broiler starter, broiler starter and broiler finisher and selected feed ingredients like maize, de-oiled rice bran, groundnut oil cake, soybean meal, broken rice and de-oiled sunflower cake that were brought by owners to Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal for a period of one year from 17th october 2019 to 16th october 2020 for testing.

The samples collected were analysed and the data was collected season wise viz., North East monsoon period (falling from October 2019 - January 2020), Non monsoon period (falling between February 2020 - May 2020) and South West monsoon period (falling between June 2020 - October 2020) and the total sample studied accounts to 1085, 682 and 1003 respectively.

Surveillance of poultry feed and feed ingredients for AFB1 contamination

Sampling

The routine poultry feed ingredients and feed materials brought by owners themselves to the Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Veterinary College and Research Institute, Namakkal were utilized for the study.

Method of estimation

AFB1 screening was done by the standardized *in house* method of AFAQAL (modified method of Romer *et al*. (1975) and quantification by TLC).

Statitical analysis

The results were analysed for its mean and standard error values to compare the level of contamination among the samples tested (Snedecor and Cochran,1974).

Results and Discussion

The type and number of poultry feed and feed ingredients samples analyzed, the results of the range and percentage contamination with AFB1 are presented in table 1 and 2 and figure 1 and 2. This survey data indicates the presence of AFB1 contamination in all the tested feed and feed ingredients. Among the samples, highest level of contamination was noticed in groundnut oil cake, maize and de-oiled rice bran at a level more than 100 µg / kg followed by broiler pre starter, broiler starter feed, broiler finisher feed, broken rice and soybean meal during the northeast monsoon, non monsoon and southwest monsoon period.

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In the present study, poultry feed and feed ingredients were screened for the presence of AFB1 contamination. The results revealed highest level of AFB1 contamination in groundnut oil cake, maize and broiler finisher at the level of >100 µg / kg. In poultry, the maximum tolerance for AF limit in feed is 20 µg / kg (FDA, 2000). OTA contamination in this study was in the range of 20 -100 µg / kg in de-oiled sunflower cake and soybean meal. The European Commission Recommendation 2006 / 576 / EC sets the maximum tolerable level for OTA contamination in poultry feeds at 0.1 mg OTA / kg (European Commission, 2006) and these safety levels were considered for evaluating the degree of contamination of AFB1 in the feed. Poultry feed and feed ingredients are prone for fungal growth during different stages of the manufacturing process *viz.*, during production, processing, transportation and storage. Hence to maintain the quality of the feed prepared, the quality control programme demands the identification of mycotoxins as an essential method which will enable to find out the fungal species and the common contaminant (Codex, 2001).

FAO (2001) provides a manual on application of Hazard Analysis and Critical Control Point (HACCP) techniques for mycotoxin prevention and control. Either preventing contamination or minimizing the toxicity of mycotoxins in feeds can improve the supply chain management of the cereal crops (Kabak *et al*., 2006). Sarathchandra and Muralimanohar (2011) screened the livestock feed and feed ingredients for the presence of AFB1, citrinin, penicillic acid, T-2, OTA and zearalenone. Their study revealed high occurrence of citrinin followed by AFB1 and OTA and suggested for sustained monitoring of the commodities and to evolve policies which discourage the marketing of toxin contaminated feeds as existing in the developed countries.

In this study, AFB1 contamination was found throughout the year. Moreover, the substrate with high protein content was prone for high level of contamination. Guerre *et al*. (2016) has mentioned the type of the substrate and climatic conditions are the main factors which influence toxin production. In warm humid subtropical and tropical conditions, crops are more prone for contamination by *Aspergillus flavus and A. parasiticus* species, and in temperate regions by *Fusarium verticillioides* resulting in the formation of AF. Toxin production is influenced not only by the geographical location but also by the annual variations in rainfall and temperature (Streit *et al*., 2012).

Hence, surveillance is very essential at every stage, irrespective of the season to identify the level of occurrence in feed and feed ingredients. Sireesha *et al.* (2017) examined commercial poultry feed samples and confirmed the presence of *A. flavus* and detected AFB1 which was below the permissible safe limits for consumption and health. The study suggested good manufacturing practices adopted by the commercial poultry feed manufacturers during procurement, handling, storage and processing of feed ingredients to prevent AF contamination.

Table 1. Surveillance of AFB1 contamination in poultry feed and feed ingredients (from October 2019 to October 2020)

Poultry feed and feed ingredients	October 2019 - January 2020 / NorthEast Monsoon Period				February 2020 - May 2020 / Non Monsoon Period				June 2020 - October 2020 / SouthWest Monsoon Period			
	Samples received	< 20 (µg / kg)	20-100 (µg / kg)	>100 (µg / kg)	Samples received	< 20 (µg / kg)	20-100 (µg / kg)	>100 (µg / kg)	Samples received	< 20 (µg / kg)	20-100 (µg / kg)	>100 (µg / kg)
Poultry Feed												
Pre broiler starter	34	32	2	0	6	6	0	0	32	32	0	0
Mean level of toxin (µg / kg)		10.24 ± 1.21	44.26 ± 0.01	-		9.26 ±0.45	-	-		13.22 ± .25	-	-
Broiler starter	28	23	5	0	7	7	0	0	25	24	1	0
Mean level of toxin (µg / kg)		12.13 ± 0.18	56.12 ± 0.16	-		8.22 ±0.39	-	-		14.46 ± .22	43.0 ± 0.39	-
Broiler finisher	37	35	1	1	16	16	0	0	32	30	2	0
Mean level of toxin (µg / kg)		15.43 ± 1.20	35.0 ± 0.0	102.0 ± 0.0		10.16 ± 14	-	-		10.15 ± 1.2	52.16± .01	-
Feed Ingredients												
Broken rice	24	24	0	0	6	6	0	0	8	8	0	0
Mean level of toxin (µg / kg)		10.2± 1.18	-	-		11.23 0.22	-	-		9.80 ± 0.36	-	-
De-oiled sunflower cake	297	289	8	0	221	215	6	0	229	224	5	0
Mean level of toxin (µg / kg)		15.30 ± 2.24	35.26± 0.32	-		12.50±3.54	42.47 ± 0.32	-		10.20 ± .78	38.15± 0.14	-
Maize	173	149	14	10	225	221	1	3	259	224	28	7
Mean level of toxin (µg / kg)		16.0± 3.86	41.2± 0.66	120.34 ± 35		11.2 ± 3.58	30.24 ± 0.0	117.22 ± 0.14		13.4 ± 3.46	56.22± 1.16	126.0 ±0.36
Groundnut oil cake	178	24	61	93	56	6	26	24	106	11	33	62
Mean level of toxin (µg / kg)		16.54 ± 0.2	97.16± 1.82	164 .12 ± 2.4		15.36 ± 0.58	82.45 ± 1.16	152.14 ± 0.00		12.0 ± 0.12	65.2 ± 0.2	170.3 ± 1.94
Soybean meal	223	223	0	0	75	75	0	0	206	206	0	0
Mean level of toxin (µg / kg)		9.36± 3.24	-	-		7.25 ± 2.18	-	-		8.50 ± 2.62	-	-
De-oiled rice bran	91	79	12	0	70	69	1	0	106	99	5	2
Mean level of toxin (µg / kg)		11.16 ± 2.54	26.65 ± 0.48	-		12.2 ± 1.94	25.2 ± 0.0	-		10.14 ± 2.99	44.2 ± 0.14	110.0 ± 0.01

Table 2. Percentage of samples contaminated with AFB1 in poultry feed and feed ingredients (from October 2019 to October 2020)

Poultry feed and feed ingredients	October 2019 - January 2020 / North East Monsoon Period				February 2020 - May 2020 / Non Monsoon Period				June 2020 - October 2020 / South West Monsoon Period			
	Samples received	< 20 (µg / kg)	20 -100 (µg / kg)	>100 (µg / kg)	Samples received	< 20 (µg / kg)	20 - 100 (µg / kg)	>100 (µg / kg)	Samples received	< 20 (µg / kg)	20 - 100 (µg / kg)	>100 (µg / kg)
Pre-broiler starter	34	94.12	5.88	0.00	6	100.00	0.00	0.00	32	100.00	0.00	0.00
Broiler starter	28	82.14	17.86	0.00	7	100.00	0.00	0.00	25	96.00	4.00	0.00
Broiler finisher	37	94.59	2.70	2.70	16	100.00	0.00	0.00	32	93.75	6.25	0.00
Broken rice	24	100.00	0.00	0.00	6	100.00	0.00	0.00	8	100.00	0.00	0.00
De-oiled sunflower cake	297	97.31	2.69	0.00	221	97.29	2.71	0.00	229	97.82	2.18	0.00
Maize	173	86.13	8.09	5.78	225	98.22	0.44	1.33	259	86.49	10.81	2.70
Groundnut oil cake	178	13.48	34.27	52.25	56	10.71	46.43	42.86	106	10.38	31.13	58.49
Soybean meal	223	100.00	0.00	0.00	75	100.00	0.00	0.00	206	100.00	0.00	0.00
De-oiled rice bran	91	86.81	13.19	0.00	70	98.57	1.43	0.00	106	93.40	4.72	1.89

Figure1. Prevalence of AFB1 (µg / kg) contamination in broiler feed (from October 2019 to October 2020)

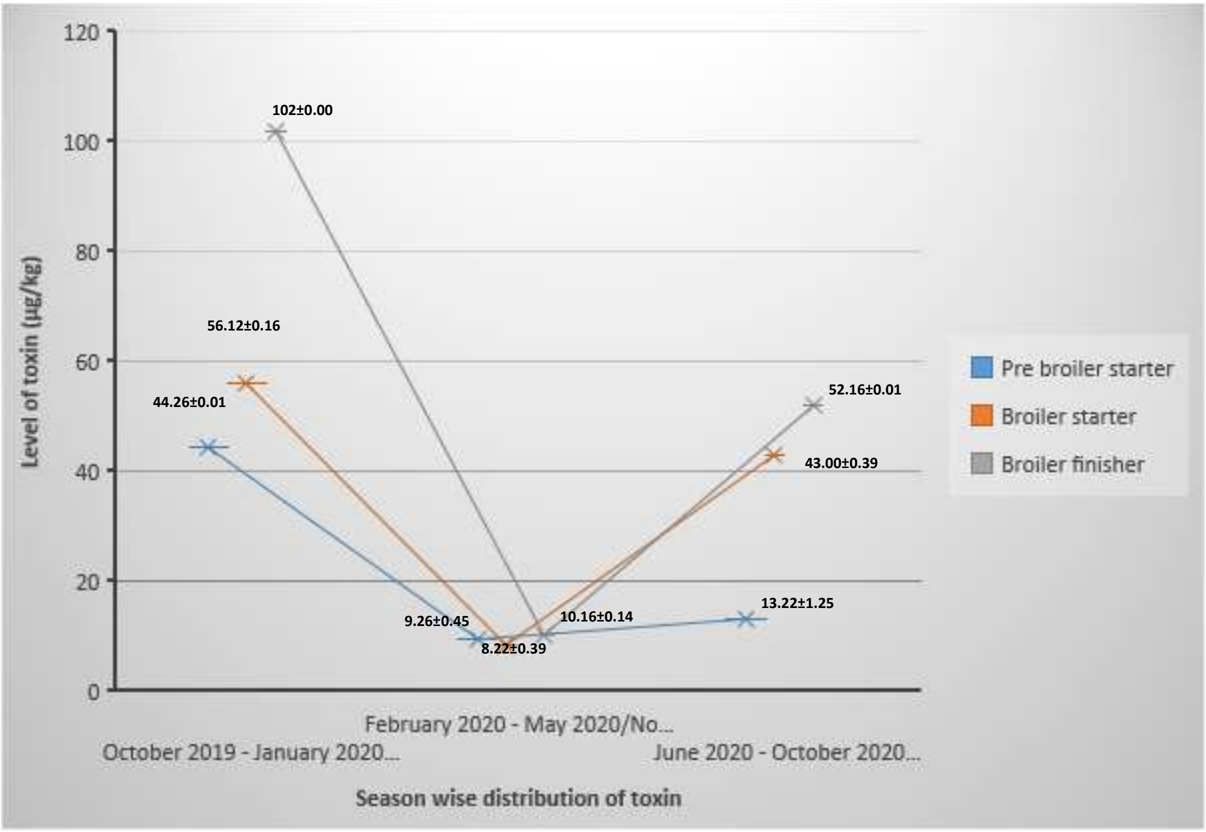
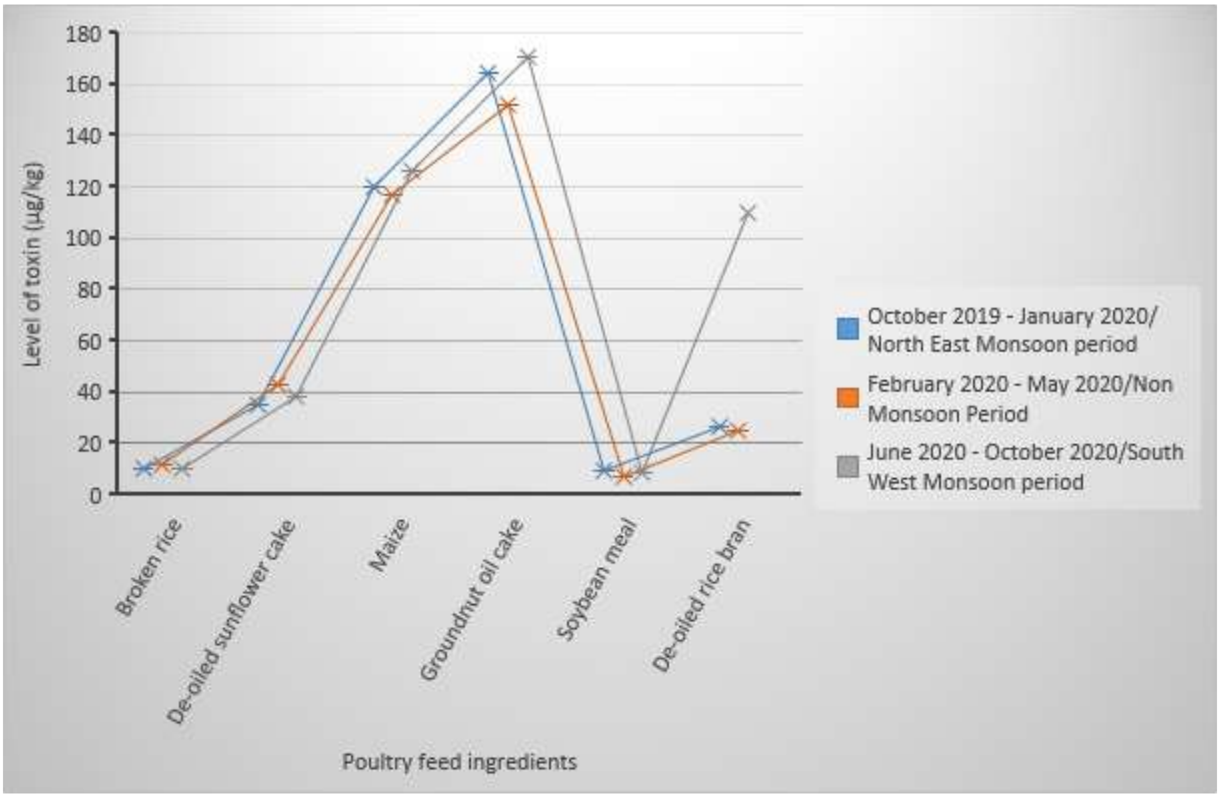


Figure 2. Prevalence of AFB1 (µg / kg) contamination in poultry feed ingredients (Oct 2019 to Oct 2020)



Our results are in accordance with the above discussions, wherein it can be declared that despite following good manufacturing practices, the method of identification of mycotoxins in poultry feed and feed ingredients is highly demanding at every stage starting from harvest of the raw material in the field, proceeding to the step of feed processing, storage and before use in the livestock and poultry feed. Also, there should be a periodical detection which gives the seasonal impact on the prevalence of mycotoxin in feed.

Hence, farmers can be advised to follow proper harvesting strategies for the ingredients most prone for contamination, to adopt efficient manufacturing of feed without contamination and to practice secured storage practices during the rainy seasons. This will aid to reduce the contamination and follow the guidelines of the international agencies involved in fixing standards. Overall, the feed supply chain can be maintained and can ensure feed safety to livestock and poultry by way of preventing and controlling the mycotoxin levels in the feed materials and thereby to human also.

Conclusions and applications

Poultry feed and feed ingredients are highly prone for fungal contamination**.** Following good agricultural practices and implementing proper safety measures during storage will ensure poultry feed and feed ingredients with minimum level of mycotoxin contamination. However, strict regulatory protocols like periodical screening will enable to supply quality feed and feed ingredients into the market.

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Conflict of Interest- The authors declare that they have no conflict of interest.

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