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Study on the analysis of levels of aflatoxins in different poultry feed raw material across different seasons in Tamil Nadu

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Abstract

The present study aimed to analysis the aflatoxin B₁ contamination levels in commonly used poultry feed ingredients during various seasons in Tamil Nadu, India. A total of 216 samples were collected between 2022 and 2023 from a commercial feed mill. The energy feed ingredients (maize, bajra, deoiled rice bran, wheat and broken rice) and protein feed ingredient (maize gluten) were analysed for aflatoxin B₁ contamination levels. The Romer's method was used to determine aflatoxin and it is expressed as parts per billion (ppb). The General Linear Model (GLM) showed season and feed ingredient had a highly significant ($p < 0.001$) effect on aflatoxin B₁ levels. Contamination levels were highest in the Northeast monsoon (20.17 ± 3.56 ppb) followed by the Southwest monsoon (16.94 ± 2.24 ppb), with lower values in winter (10.96 ± 1.08 ppb) and summer (10.33 ± 1.01 ppb). Maize gluten and deoiled rice bran were more vulnerable to contamination, with maize gluten exhibiting the highest level of 78.00 ± 6.00 ppb during the monsoon season. The season and ingredient interaction was also statistically significant ($p < 0.001$), suggesting that environmental factors play a significant role in the risk of contamination. The research demonstrates the importance of seasonal testing and better storage conditions to minimise aflatoxin contamination in poultry feed.

Keywords: Aflatoxin B₁, Poultry feed ingredients, Feed mill, Seasonal variation, GLM

1. Introduction

Poultry is one of the fastest growing livestock industries in India due to the growing demand for protein rich food and rapid commercialisation of feed manufacturing. (Churchil, 2022) [2] There has been significant growth in the animal feed industry in India, with poultry being a major contributor owing to its cost effective nature and efficiency as a protein source (Alice Mitchell, 2015) [1]. Feed represents the largest share of the cost of production and is mostly made up of cereals and oil seed meal. But, these feed ingredients are prone to fungal invasion resulting in the production of toxic secondary compounds called mycotoxins.

Aflatoxin B₁ (AFB₁) are the most toxic and economically important mycotoxins that affect poultry health and performance. (Ghimpețeanu, Tolescu, & Militaru, 2012) [3] Aflatoxins are primarily produced by *Aspergillus flavus* fungus under environmental conditions such as warm and humid weather, which is typical of tropical and subtropical climates. Previous studies under warm climate conditions have adequately shown the potential for increased risk of aflatoxin contamination of feed ingredients.

Aflatoxin contamination can happen at various levels such as pre harvest, post harvest, storage and during feed processing. The presence of aflatoxins in feed ingredients can cause a range of poultry health issues, such as poor growth, immunosuppression, feed efficiency and susceptibility to diseases (Sireesha, Keerthana, Sarathchandra, & Velazhahan, 2017) [8]. Additionally, aflatoxins can be transferred to humans through animal products like meat and eggs, presenting risks to human health.

Maximum levels of aflatoxin contamination in feed and food have been set globally by regulatory bodies to reduce potential risks. The suggested maximum level for AFB₁ in poultry

feed is 20 µg/kg, but the regulations differ internationally depending on risk assessment and regulatory strategies taken into account. However, it's difficult to eradicate aflatoxins entirely due to their natural presence and environmental factors.

The seasons can have a significant impact on aflatoxin contamination. Weather conditions like rainfall, humidity and temperature impact the growth and production of toxins (Jayapradha *et al.*, 2026) [5]. A few studies have shown higher incidence of aflatoxin during the monsoon than in the dry season in India. In India, previous studies have also reported substantial seasonal change in aflatoxin content in feed ingredients, with a higher incidence during monsoons in Tamil Nadu. The recent study by (Priya, Jagadeeswaran, & Natarajan, 2023) [6] revealed that aflatoxin B1 contamination is year-round with a higher prevalence during Northeast and Southwest monsoons, especially in ingredients like maize and deoiled rice bran. These studies provide further evidence of the impact of environmental conditions on occurrence.

Along with environmental factors, the vulnerability of feed ingredients also differs. Protein and oil rich ingredients such as maize gluten, groundnut cake and deoiled rice bran are highly susceptible to fungal infestation, which leads to the production of aflatoxin. The combined effect of ingredient type and season also plays a role in contamination, requiring comprehensive monitoring.

While various studies have reported on aflatoxin contamination of feed ingredients, there is a lack of systematic research on season ingredient interactions under field conditions in particular agro climatic zones, such as Tamil Nadu.

Hence, the current study was conducted to assess the aflatoxin B₁ contamination in major poultry feed ingredients over different seasons in Tamil Nadu using a General Linear Model (GLM) approach. The results contribute to an understanding of seasonal risks and ingredient vulnerability and the development of risk management strategies to improve feed safety, prevent animal and human health issues and improve farm productivity.

2. Materials and methods

2.1 Study Area

The study was conducted at a commercial feed mill located in Udumalpet, Tamil Nadu, India. A total of 216 samples were collected over two years (2022-2023). The study considered of two main factors *viz.*, season (winter, summer, southwest monsoon, northeast monsoon) and feed ingredients (maize, bajra, deoiled rice bran (DORB), wheat, broken rice, maize gluten). Aflatoxin B₁ levels were determined using Romer's analytical method and results were expressed in parts per billion (ppb).

2.2 Aflatoxin Extraction and Clean-Up (TLC Method)

Aflatoxin extraction was carried out by adding 40 ml of distilled water and 60 ml of acetone to 10 g of ground sample. The mixture was stirred for 3 minutes and filtered through whatman filter paper. An aliquot (30 ml) of the filtrate was treated with 30 ml of NaOH and 6 ml of ferric chloride solution in one beaker (A) and 0.6 g of cupric carbonate was added in another beaker (B). The mixtures were shaken vigorously to facilitate gel formation and then filtered again. Subsequently, 40 ml of filtrate was transferred into a 250 ml separating funnel. To this, 40 ml of 0.03% H₂SO₄ and 10 ml chloroform were added and mixed gently. In a separate funnel, 40 ml of 0.02 M of KOH containing 1% KCl was prepared. The chloroform layer was collected and passed through anhydrous Na₂SO₄ to remove moisture. The extract

was evaporated to near dryness using a water bath and reconstituted in chloroform for thin-layer chromatography (TLC) analysis. (Romer, 1975)

2.3 Thin Layer Chromatography (TLC) Analysis

TLC plates were developed using a solvent system consisting of chloroform:acetone (9:1). For spotting, 200 µL chloroform was added to a vial and 15 µL of sample extract was applied onto the TLC plate. Plates were developed, dried and visualized under a UV cabinet for aflatoxin detection.

2.4 Quantification of Aflatoxin

Aflatoxin concentration was calculated using the following formula:

$$\text{Aflatoxin (ppb)} = \frac{\text{Comparison} \times \text{Concentration} \times \text{Dilution}}{\text{Sample Volume (15 } \mu\text{L)} \times \text{Effective Weight (1.714)}} \times 1000$$

2.5 Statistical Analysis

All the data were analyzed using the General Linear Model (GLM) procedure in Minitab (Version 19). Season and ingredient type were treated as fixed effects and their interaction was included in the model. Statistical significance was determined at $p < 0.01$. Post-hoc comparisons were performed using Tukey's test.

3 Results and Discussion

3.1 Seasonal variation in Aflatoxin contamination (2022-2023)

The results clearly demonstrated that aflatoxin B₁ contamination in poultry feed ingredients varied significantly across seasons ($p < 0.001$). In both years, higher contamination levels were consistently observed during the monsoon periods, particularly the Northeast monsoon (NE-M), followed by the Southwest monsoon (SW-M), while comparatively lower levels were recorded during winter and summer seasons (Tables 1-3).

In 2022 (Table 1), the NE-M season exhibited the highest aflatoxin levels in several ingredients, mainly maize gluten (78.00±6.00 ppb) and broken rice (19.33±7.42 ppb). Similarly, during SW-M, higher levels of contamination was observed in maize gluten (21.67±6.33 ppb) and broken rice (15.67±0.67 ppb). In contrast, winter and summer seasons showed lower contamination levels across most ingredients.

A similar trend was observed in 2023 (Table 2), NE-M recorded the highest values across all ingredients, with maize (21.33±8.41 ppb), DORB (14.00±4.03 ppb) and maize gluten (25.50±9.54 ppb) showing considerable increases. The SW-M season also showed higher levels of contamination, particularly in maize gluten (28.00±10.90 ppb). These findings indicate strong seasonal consistency across years.

The overall mean values (Table 3) further confirmed that NE-M (20.17±3.56 ppb) and SW-M (16.94±2.24 ppb) had significantly higher aflatoxin levels compared to winter (10.96±1.08 ppb) and summer (10.33±1.01 ppb). This trend reflects the influence of environmental conditions such as high humidity, temperature fluctuations and moisture availability during monsoon seasons favouring fungal growth and aflatoxin production. (Guerre, 2016) revealed that both the nature of the substrate and prevailing climatic conditions plays a key role in toxin production.

3.2 Ingredient wise susceptibility to Aflatoxin contamination

Across both years and seasons, maize gluten (MG) and deoiled rice bran (DORB) were found to be more susceptible to aflatoxin contamination compared to other ingredients.

Significant differences ($p < 0.001$) were also observed among all feed ingredients.

Maize gluten consistently recorded the highest contamination levels, particularly during NE-M (41.56 ± 9.92 ppb) followed by SW-M (25.89 ± 7.32 ppb). This could be attributed to its high protein content and hygroscopic nature making it more prone to fungal invasion under favorable environmental conditions. Among energy ingredients, DORB also exhibited elevated contamination levels, especially during monsoon seasons, due to its residual oil content and susceptibility to moisture absorption. Broken rice and maize showed moderate contamination levels, whereas wheat generally recorded lower values across seasons. Bajra showed intermediate variability but did not exhibit extreme contamination levels compared to maize gluten.

3.3 Interaction between Season and Ingredient

The interaction between season and ingredient was found to be significant ($p < 0.001$) indicating that the extent of aflatoxin contamination in a given ingredient is highly dependent on seasonal conditions. For instance, maize gluten showed minimal contamination during winter but exhibited a drastic increase during NE-M (78.00 ppb in 2022) highlighting the combined effect of substrate susceptibility and environmental conditions. Similarly, broken rice and DORB showed marked increases during monsoon seasons, whereas wheat remained relatively stable across seasons. This interaction emphasizes the need for ingredient-specific storage and handling strategies depending on seasonal risk factors. (Sireesha *et al.*, 2017) [8] analyzed commercial poultry feed samples and confirmed the presence of *Aspergillus flavus*, below the permissible safety limits for consumption for birds.

Table 1: Effect of different seasons of 2022 on aflatoxin level in energy and protein ingredients

Season	Bajra	Maize	DORB	BR	Wheat	MG
Winter	14.67 ± 2.85^b	23.67 ± 6.17^a	4.00 ± 3.51^c	10.33 ± 1.33^b	9.67 ± 0.67^a	2.67 ± 1.20^c
Summer	11.67 ± 2.67^c	9.33 ± 0.88^c	14.00 ± 1.00^a	7.00 ± 1.53^c	8.33 ± 0.67^b	17.33 ± 9.24^b
SW-M	13.67 ± 0.67^b	10.67 ± 1.67^b	13.67 ± 0.67^a	15.67 ± 0.67^a	10.33 ± 0.67^a	21.67 ± 6.33^b
NE-M	16.67 ± 0.88^a	10.67 ± 4.41^b	7.00 ± 1.00^b	19.33 ± 7.42^a	2.00 ± 0.58^c	78.00 ± 6.00^a

Data collected in triplicate and the values represented as Mean $\pm$ SE

^{a,b,c} Means within a column with no common superscript differ significantly ($p < 0.05$)

Table 2: Effect of different seasons of 2022 on aflatoxin level in energy and protein ingredients

Season	Bajra	Maize	DORB	BR	Wheat	MG	BR
Winter	12.83 ± 1.25^b	15.17 ± 1.47^b	7.50 ± 2.43^b	12.83 ± 1.51^b	9.67 ± 1.71^b	8.83 ± 1.97^c	12.83 ± 1.51^b
Summer	6.50 ± 1.53^c	13.00 ± 1.08^b	6.33 ± 2.87^b	10.50 ± 2.16^b	8.50 ± 2.07^b	9.33 ± 3.06^c	10.50 ± 2.16^b
SW-M	12.50 ± 1.54^b	16.00 ± 3.83^b	12.17 ± 3.19^a	15.50 ± 3.04^b	13.67 ± 2.56^a	28.00 ± 10.90^b	15.50 ± 3.04^b
NE-M	16.33 ± 1.20^a	21.33 ± 8.41^a	14.00 ± 4.03^a	23.00 ± 9.45^a	14.00 ± 4.41^a	25.50 ± 9.54^a	23.00 ± 9.45^a

Mean $\pm$ SE of 6 observations are presented ^{a,b,c} Means within a column with no common superscript differ significantly ($p < 0.05$)

Table 3: Overall mean aflatoxin levels across ingredients

Season	Bajra	Maize	DORB	MG	Wheat	BR	Overall Mean
Winter	15.00 ± 1.38	16.22 ± 2.53	6.22 ± 2.04	6.78 ± 1.63	9.67 ± 1.08	11.89 ± 1.13	10.96 ± 1.08^c
Summer	10.11 ± 1.80	11.78 ± 1.09	10.00 ± 2.31	12.00 ± 3.77	8.67 ± 1.33	9.44 ± 1.64	10.33 ± 1.01^c
SW-M	14.00 ± 0.88	14.78 ± 2.70	12.67 ± 2.48	25.89 ± 7.32	12.44 ± 2.03	17.89 ± 2.48	16.94 ± 2.24^b
NE-M	16.67 ± 0.88	17.67 ± 6.60	11.78 ± 2.80	41.56 ± 9.92	10.56 ± 3.81	22.78 ± 7.41	20.17 ± 3.56^a

Conclusion

The current study confirms that level of aflatoxin B₁ in poultry feed ingredients is affected by season and ingredient used. The monsoon seasons, especially the Northeast monsoon, had the highest contamination levels, which highlights the influence of humidity and moisture in the growth of aflatoxin producing fungi. Maize gluten and deoiled rice bran were found to be most common and sometimes exceeded the maximum tolerable levels of contamination. The season and the ingredient interaction suggest that risk is both seasonal and ingredient specific. It is concluded that seasonal storage practices, monitoring and quality control, particularly during the monsoon seasons, should be adopted for the good practices of feed safety management and reduce contamination of the animal and human food chain with aflatoxins.

Conflict of Interest

Not available

Financial Support

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