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Research Paper

Aflatoxins in commonly used feed raw materials: a quantitative analysis

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Abstract: Aflatoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* are known to be carcinogenic and mutagenic. Aflatoxins concentration (AC) in feed raw materials such as maize, wheat, peanut and rice by-products can exceed the threshold levels resulting in reduced quality of feed produced. This study aimed to quantify the AC and its association with the proximate composition (PC) (moisture, crude protein, ether extract, crude fibre and ash) of common feed raw materials. Two-year (2021-2022) data of AC and PC of commonly used feed raw materials were extracted from a database of one of the largest concentrate feed manufacturers in Sri Lanka. The data were then analysed using R software version 4.01, and the associations between PC and AC were quantified using correlation analysis and principal component analysis (PCA). The number of PCA components was determined using both Eigenvalues and Monte Carlo PCA simulation. The highest crude protein was found in Meat & Bone Meal (50.3±1.8%) and the lowest in Palm Kernel Meal (17.8±1.5%) of the protein sources used in feed formulation. The Dried Distillers Grains had the highest moisture content (14.9±1.8%) and Meat & Bone Meal had the lowest moisture content (3.4±2.0 %). Overall, the AC of most of the raw materials used in feed processing exceeded the threshold of 20μg/kg with the lowest level being observed in Boll Rice (4.9±1.2μg/kg) and the highest (55.5±58.3μg/kg) in Dried Distillers Grains. The correlation coefficients ranged from -0.68 to 0.57 and aflatoxin concentrations were generally poorly associated with feed nutrients except for moisture content (r=0.35). Therefore, these findings suggest that the aflatoxin concentration in commonly used feed raw materials increases when moisture content increase. This suggests that proper drying of raw materials should be encouraged prior to storage and feed processing to reduce the aflatoxins concentration in feed raw materials.

Keywords: Aflatoxins, Concentrate feeds, Feed raw materials, Nutritional quality

Introduction

Aflatoxins are a group of mycotoxins mainly produced by *Aspergillus flavus* & *Aspergillus parasiticus* and are known to be carcinogenic and mutagenic to livestock animals and human when ingested beyond the threshold level (20μg/kg). There are four main types of aflatoxins (B1, B2, G1 and G2) and aflatoxin B1 is the most toxic, carcinogenic of all and the predominant mycotoxin in agricultural produces in the world (Omara *et al.*, 2021). Aflatoxin toxicity, called aflatoxicosis, can decrease the growth rates of animals and milk production and lead to poor health, resulting in severe economic losses to animal husbandry globally (Dhanasekaran *et al.*, 2011). Therefore, it is a global concern to mitigate aflatoxicosis, particularly in the tropics and subtropics including Sri Lanka where the warm and humid climate conditions favour the growth of aflatoxin-producing fungi in agricultural produce used to feed livestock animals (Kana *et al.*, 2013).

Feed raw materials such as maize, wheat, rice by-products, peanut, cottonseed and oil products used in concentrate feed production can contain aflatoxins beyond threshold levels resulting a low quality of feed produced (Sun *et al.*, 2011). In Sri Lanka, most agricultural produce, especially peanut, rice & maize and imported raw materials have been reported to contain high amount of aflatoxins, however, sufficient studies have not been carried out to quantify the aflatoxins concentrations in both locally produced and imported raw materials used in concentrate feed production. This study aimed to quantify the concentrations of aflatoxins in commonly used raw materials and evaluate the association between aflatoxins concentrations and the proximate composition of feed raw materials.

Materials and Methods

Study Location and Design

This study utilized secondary data spanning two years (2021–2022), obtained from the central database of a prominent feed manufacturing company located in Colombo, Western province of Sri Lanka. The company specializes in producing cattle, poultry, and swine feed, with a monthly production capacity of 15–20 tons. The dataset included the proximate composition (moisture, crude protein, ether extract, crude fibre, and ash) and aflatoxin concentration levels of feed raw materials analysed both upon receipt and during storage. Data included the analysis for both imported feed raw materials from India, Pakistan, Ukraine, Italy, Germany & the USA and locally produced raw materials.

Storage Conditions and Sample Collection

Raw materials were sampled according to the company's quality assurance protocols, employing a stratified random sampling method to ensure representation across different suppliers and batches. Samples were collected daily upon receipt and weekly during storage. Raw materials were stored in dry open warehouse conditions with temperature conditions maintained between 27–33°C and relative humidity (RH) levels ranging from 65–70%. These conditions were monitored routinely and logged as part of the feed manufacturer's quality assurance procedures. The storage duration varied depending on the type of raw materials – seasonal raw materials such as maize are stored for up to about six (6) months, regular raw materials such as rice bran and rice polish are stored for a month while imported raw materials such as soybean meal and mechanical deboned meat are stored for about three (3) months.

Analytical Procedures

Aflatoxin concentrations were measured using enzyme linked immune absorbent assay (ELISA) following AOAC International methods, ensuring precision and reliability. Proximate composition was determined using standard protocols, including oven-drying for moisture content, the Kjeldahl method for crude protein, Soxhlet extraction for ether extract, and ash content determination via muffle furnace incineration at 550°C. All analyses were conducted in triplicate to enhance data accuracy and repeatability.

Statistical Analysis

Data were analysed using R software version 4.01 (open-source software under a creative commons license provided under the CRAN project). Descriptive summary statistics were done to generate means and standard deviations in order to test for the variability of the data. Outliers were identified and removed from the database. To evaluate the association between proximate composition and aflatoxin concentrations of feed raw materials, correlation analysis and principal component analysis (PCA) were performed using the FactomineR R extension package. The number of principal components was determined using both Eigenvalues and Monte Carlo PCA simulation. Generally, principal components with Eigenvalues less than 1.0 were not considered.

Results and Discussion

Aflatoxin concentrations in commonly used feed raw materials

The aflatoxin concentrations of 81% of the raw materials used in concentrate feed formulation were above the maximum limit of 20μg/kg recommended by the United States Food and Drug Administration (Table 1).

Dried distiller's grains with solubles (DDGS) had the overall highest aflatoxin concentration. Pule Addison *et al.* (2018) also reported that total aflatoxins in DDGS ranged from 15 - 70μg/kg while Rodrigues (2011) reported that aflatoxin concentrations in

DDGS can even reach to a maximum of 89µg/kg. Further, Zhang and Caupert (2012) reported that DDGS used in the USA had mycotoxin concentrations three times higher than that of the grains. DDGS is a by-product of ethanol

Table 1. Aflatoxins concentrations in common feed raw materials

Raw material	Origin	Aflatoxin (Mean ± SD) (µg/kg)
DDGS	USA	55.5±58.3
Coconut Poonac	SL	26.4±11.9
Red Rice Polish	SL	24.8±13.9
Maize	IND	24.0±12.3
Maize	SL	23.6±27.4
Rice Parboiled Polish	SL	22.6±15.2
Rice Polish (White)	SL	22.0±25.5
Rice Broken	SL	12.0±10.9
Wheat	UKR	9.0±11.2
Rice Bran	SL	6.6±3.7
Maize	PAK	6.6±2.1
Soybean Meal High Protein	USA	6.0±3.5
Boll Rice	SL	4.9±1.2

DDGS: Dried distiller's grains with solubles; USA: United States of America; SL: Sri Lanka; IND: India; UKR: Ukraine; PAK: Pakistan

production, therefore, it can contain a high amount of moisture as we found in this study resulting in higher production of aflatoxins during storage. It is also known that aflatoxins do not get destroyed during ethanol production, therefore, it is probable that aflatoxins contained in grains used for ethanol production could eventually accumulate in DDGS resulting in these high concentrations.

The aflatoxin concentrations in maize from India and Sri Lanka were also higher than the recommended threshold levels (Table 1). Williams *et al.* (2002) reported that maize is generally very susceptible to aflatoxins, therefore, known to have high concentrations of aflatoxins. Jayaratne *et al.* (2020) further suggested that maize grown in some areas of Sri Lanka are exposed to aflatoxins and thus gets contaminated with very high concentrations of aflatoxins. Maize grains used as raw materials for concentrate feed production could either get contaminated with aflatoxins on the field, during harvest or during storage. Though very limited studies have been conducted on the soil aflatoxins concentration in Sri Lanka, Jayaratne *et al.* (2020) found soil aflatoxin B1 concentrations in a major maize growing district to be as high as 300µg/kg which implies a positive association between soil aflatoxins concentration and that of the maize grains. Improper drying and storage conditions of maize grains can be another possible reason for the high aflatoxin contamination, however the storage and atmospheric conditions at the dry open warehouses are ideal and should not promote the growth of aflatoxins under normal circumstances. Maize is generally known to be very susceptible to fungal attack when the moisture content is high, especially by *Aspergillus* & *Fusarium* and other aflatoxins-producing fungi that belong to *Aspergillus spp.* (Tefera, 2012).

Various rice by-products of Sri Lankan origin had aflatoxin concentrations over the threshold levels except for boll rice, rice bran and broken rice (Table 1). Not many studies have been conducted on aflatoxins in rice by-products in Sri Lanka, however, Bandara *et al.* (1991) reported that parboiled rice had up to a maximum of 185µg/kg aflatoxin B1 concentrations. Aflatoxins can survive during the intense rice milling process and therefore can be abundantly present in the milling by-products such as polish and bran as we observed in this study.

Generally, raw materials such as maize, rice, wheat, and bakery meal that are rich in carbohydrates are a good growth medium for *A. flavus* development, therefore, aflatoxins can be expected in such feed raw materials although the concentrations can be below the threshold if properly managed. Further, the warm & humid climate and poor storage conditions may have resulted in high growth of aflatoxins producing fungi in feed raw materials.

This study did not consider other factors such as temperature, relative humidity, storage time and storage conditions which may have contributed to the high concentrations of aflatoxins observed in these feed raw materials. Future studies are recommended to evaluate the effect of such factors and samples of raw materials from various parts of the country should be analysed to understand the dynamics of aflatoxins concentration in feed raw materials used in concentrate feed manufacturing in Sri Lanka.

Proximate composition of various feed raw materials

Maize from India and Pakistan had moisture above the recommended levels for feed manufacturing (14%) while maize from Sri Lanka had less than 14% of moisture (Table 2). Maize imported from India and Pakistan and produced in Sri Lanka had approximately 7% of crude protein (CP). Wheat from India and Ukraine did not differ much in their proximate compositions. The proximate composition of rice by-products showed a level of consistency with previous studies (Mujahid *et al.*, 2003).

Meat and bone meal (MBM) has been used as the main protein raw material in feed formulation at the feed mill and the CP content was slightly lower than the reported values (53 – 55% CP) by Kellems & Church (2010) (Table 3). Palm kernel meal (PKM) contained less than 20% of CP even though it was used as a protein raw material that ought to contain a minimum of 20% CP.

Soybean meal from USA, India and Pakistan showed very similar results in their proximate composition and this finding is consistent with that of Kellems & Church (2010).

Association between aflatoxin concentration and feed raw materials' proximate composition

The principal component analysis indicated that only two principal components were sufficient to explain the 73% variation (i.e. 47.55% + 25.47%) between proximate composition and aflatoxin concentrations of the feed raw materials (Figure 1), thus capturing key patterns between proximate composition and aflatoxin levels.

Table 2. Proximate composition (%) of feed raw materials (energy sources)

Raw Material	Origin	Moisture	CP	EE	CF	Ash
Maize	SL	13.1±1.7	7.8±1.2	3.7±0.8	2.7±0.4	1.3±3.4
Red Rice Polish	SL	11.5±1.0	11.0±1.0	14±2.8	8.5±0.3	6.6±1.0
Rice Broken	SL	13.1±1.2	7.7±0.8	4.2±1.9	3.6±2.3	2.3±8.0
Wheat	IND	11.3±0.9	11.6±1.0	-	-	1.5±0.5
Wheat	UKR	11.8±0.6	11.6±1.2	-	-	1.5±0.7
Maize	PAK	14.1±2.1	7.7±0.6	3.7±0.1	2.1±0.4	1.3±0.8
Bakery By-product Meal	SL	14.3±1.1	11.8±4.2	4.1±2.4	3.2±0.1	3.4±1.4
Rice Parboiled Polish	SL	9.8±1.7	9.1±2.3	7.1±3.8	9.0±0.4	9.7±3.5
Wheat Bran	SL	13.1±0.5	14.6±0.6	3.6±0.4	7.5±0.3	4.8±0.3
Rice Polish (White)	SL	11.2±1.0	10.5±0.9	11.1±3.5	8.6±0.2	6.4±0.9
Balanced Wheat Flour	SL	13.7±0.3	13.4±0.4	2.6±0.3	2.8±1.2	3.7±0.3
Rice Bran	SL	9.8±0.6	12.3±1.6	20.0±2.9	8.4±0.3	10.2±0.7
Maize	IND	14.9±1.5	7.7±0.2	3.7±0.1	2.4±0.4	1.2±0.1

CP: Crude protein; EE: Ether extract; CF: Crude fibre; SL: Sri Lanka; IND: India; UKR: Ukraine; PAK: Pakistan

Table 3. Proximate composition (%) of feed raw materials (protein sources)

Raw Material	Origin	Moisture	CP	EE	CF	Ash
Soybean Meal High Protein	USA	12.2±0.7	45.8±2.2	1.4±0.5	3.7±0.4	6.5±1.2
Meat and Bone Meal	ITA/GER	3.4±2.0	50.3±1.8	11.5±1.6	10.4±0.2	32±2.7
Soybean Meal High Protein	IND	12.3±0.5	45.2±0.6	1.1±0.2	6.4±0.5	7.5±0.7
Soybean Meal High Protein	PAK	12.3±0.6	45.4±4.6	1.0±0.2	4.7±0.2	6.4±0.1
DDGS	USA	14.9±1.8	29.3±1.6	4.9±0.5	-	5.7±0.8

Coconut Poonac	SL	7.6±1.3	25±1.8	7.9±0.4	-	4.7±0.7
Palm Kernel Meal	SL	5.2±2.3	17.8±1.5	8.6±0.6	9.4±0.4	2.5±0.7

CP: Crude protein; EE: Ether extract; CF: Crude fibre; DDGS: Dried distiller's grains with solubles; USA: United States of America; ITA: Italy; GER: Germany; PAK: Pakistan. IND: India; SL: Sri Lanka.

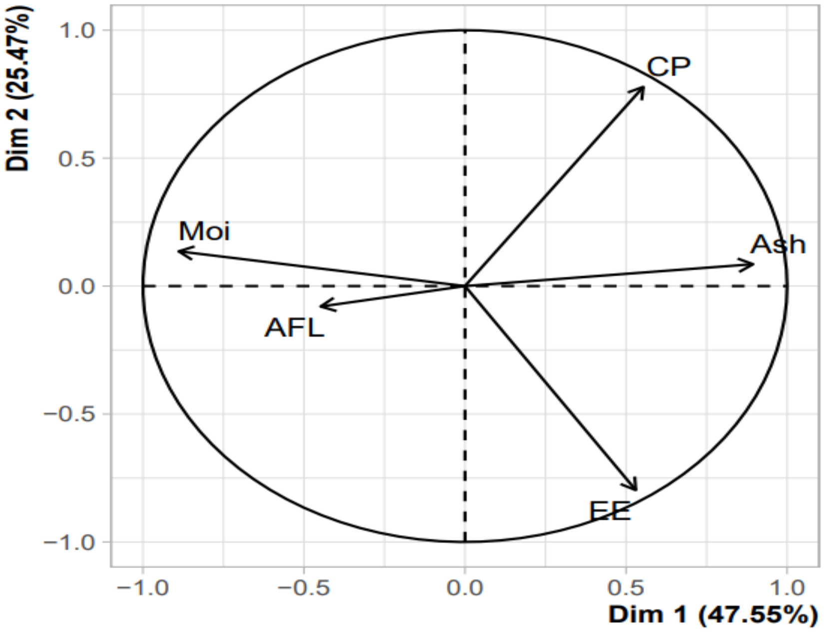

Figure 1. Principal component analysis graph showing the association between feed raw materials nutrient parameters and aflatoxins concentration

The correlation coefficients ranged from -0.68 to 0.57 and aflatoxin concentrations were generally poorly associated with feed raw materials' proximate composition except for moisture content (r = 0.35) (Figure 2). The aflatoxin concentrations of the raw materials are more positively associated with the moisture content of the raw materials than any other proximate component, therefore, aflatoxin concentrations increases as the moisture content in raw materials increases. This result is supported by the findings of several other studies which indicated that high moisture content is a predisposing factor to the growth of *A. flavus* and *A. parasiticus* and aflatoxins production (Schmidt-Heydt *et al.*, 2008; Oms-Oliu *et al.*, 2010; Bryden, 2012; Tefera, 2012; Castellari *et al.*, 2015). These advanced statistical techniques provide a solid framework for identifying high-risk raw materials and targeting them for stricter quality control measures.

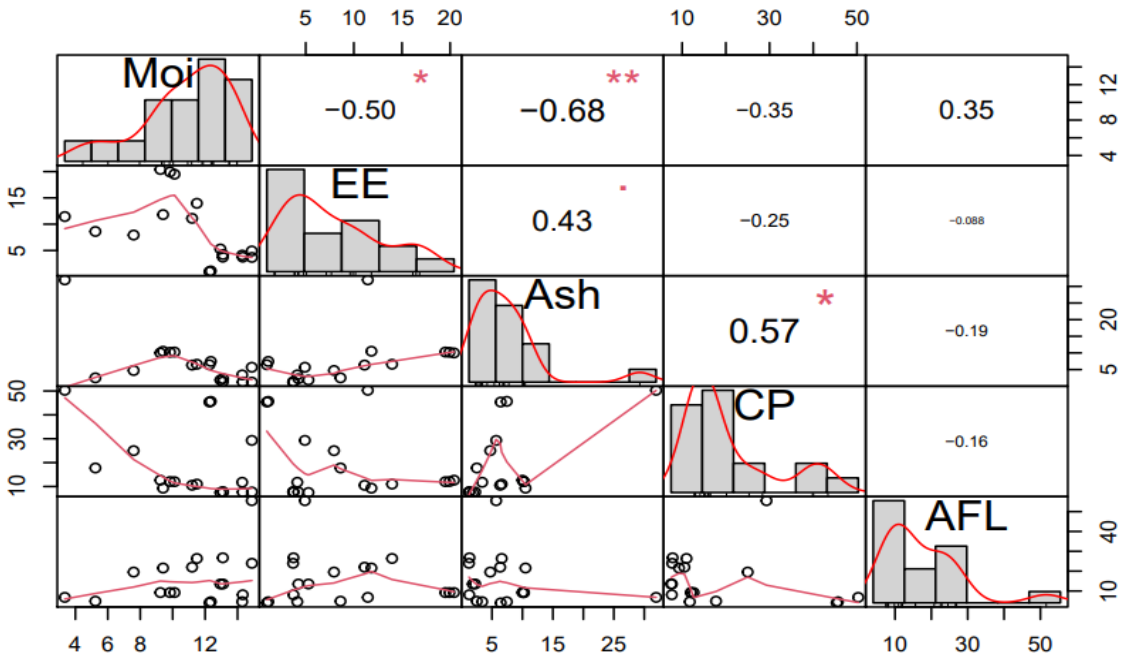

Figure 2. Correlation chart and matrix for common feed raw materials nutrients

It is expected that after harvest, grains ought to be dried properly to reduce their moisture content to less than 14% to prevent fungal growth during storage (Magan and Aldred, 2007). Most local farmers depend largely on sunlight as means to dry cereal grains and sun-drying sometimes fails to dry grains sufficiently or promptly to prevent fungal attack since it is heavily dependent on the local weather (Wagacha *et al.*, 2013; Womack *et al.*, 2014). The moisture content of most of the raw materials analysed in this study were below 14% which is the general recommended moisture level, however, it is known that *A. flavus* and *A. parasiticus can* thrive in a wide range of moisture levels and may have contributed to the increase in aflatoxins.

Abiotic factors such as temperature, relative humidity, water activity as well as insect pests of stored raw materials were not put into consideration in this study even though they might possibly have some association with aflatoxins concentration. For instance, as most feed raw materials are hygroscopic in nature and they are influenced by the relative humidity of their surrounding environment during storage which in turn affects both the water activity and moisture content over time creating a favourable environment for the growth of *Aspergillus spp.* and aflatoxins production. Therefore, further studies are recommended to evaluate the effect of such factors on aflatoxin concentrations in feed raw materials,

Conclusion

This study quantified aflatoxin concentrations in commonly used feed raw materials and evaluated their association with proximate composition. The findings revealed that 81% of the raw materials exceeded the FDA threshold of 20 µg/kg, with DDGS and maize exhibiting the highest contamination levels. There is a positive association between the moisture content of feed raw materials and aflatoxin concentrations suggesting that further drying of raw materials might be beneficial in reducing aflatoxin levels. These results provide valuable insights for feed manufacturers in tropical regions, where warm and humid climates favour fungal growth. Implementing controlled storage conditions, regular monitoring of aflatoxin levels, and adopting advanced drying technologies can significantly mitigate contamination. Furthermore, the study highlights the importance of comprehensive quality assurance frameworks that consider environmental factors and material variability. Future research should extend these findings by incorporating real-time monitoring of storage conditions, exploring the role of additional abiotic factors such as temperature fluctuations, and evaluating the long-term impact of contamination on animal productivity and health. These efforts will be crucial for advancing feed safety and aligning practices with international standards to ensure sustainable livestock production.

This study also gave insight into the status of aflatoxins in feed raw materials such as rice by-products that have not been extensively evaluated for aflatoxins in Sri Lanka. Overall, most of the commonly used feed raw materials of Sri Lankan origin have high aflatoxin concentrations beyond the maximum limit, Therefore, further studies can be conducted to evaluate the status of aflatoxins in feed raw materials collected from different districts of Sri Lanka to better control the development of aflatoxin in feed raw materials. Particularly, aflatoxin B1, the most toxic and predominant aflatoxin in raw materials in Sri Lanka, should be quantified prior to the use of raw materials in feed processing.

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