PREVALENCE OF AFLATOXIN B1 IN SELECTED FEED INGREDIENTS FROM FOUR MAJOR FEED MILLS IN IJEBU ODE AREA OF OGUN STATE

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Abstract: Mycotoxins affect nutritional and economic value of staple foods and cash crops especially in developing countries including Nigeria. Aflatoxin B1 when consumed by man or animal can cause liver cancer, reduced fertility, increased susceptibility to infections among other terrible diseases. In view of this a study was conducted to determine and compare the level and extent of aflatoxin B1 contamination of four different poultry feed ingredients namely maize, wheat offal, soya bean meal and groundnut cake obtained from 4 selected major feed mills (A, B, C and D) in Ijebu Ode, Ogun State of Nigeria. 500g samples of each ingredient were collected out of which 0.5g sample each in four replications of maize, wheat offal, soybean meal and groundnut cake were ran separately with 5g of sodium chloride (Nacl) weighed and poured in a blender jar to determine their aflatoxin B1 level. Aflatoxin B1 level was determined with ELISA (Enzyme Linked Immunosorbent Assay) reader at 450nm. The results obtained were analysed using statistical analysis software (SAS 1999) while the means were compared using Duncan multiple range test. The analysed result of aflatoxin B1 levels in all the feed ingredients showed significant differences across all the feed mills. Aflatoxin B1 concentration in maize for feedmill A, B, C & D were 25.93, 13.10, 15.28 and 15.28ppb respectively. In soybean meal, aflatoxin B1 levels were 30.48, 24.78, 20.63 and 157.85ppb respectively for A, B, C & D respectively. Aflatoxin B1 concentration for groundnut cake in A, B, C & D were 368.43, 289.28, 288.08 and 157.45ppb respectively. Wheat offal in A, B, C & D had mean values of 36.63, 49.84, 26.88 and 47.63ppb respectively. It was found from the study that in most cases, the aflatoxin B1 levels of all feed ingredients determined were above the 20ppb safe level for animal consumption. Therefore, feed ingredients in Ijebu Ode local government area and particularly from the selected feed mills during the period of the study were highly contaminated with aflatoxin probably due to poor storage facilities and long storage time. A regular and routine check on the storage facilities of these feed millers by regulatory authorities is recommended.

Key words: Aflatoxin B1, Feed ingredients, Prevalence, Feed mill, ELISA.

Introduction

Aflatoxin is a naturally occurring mycotoxin that is produced by *Aspergillus flavus* and *Aspergillus parasiticus.* They commonly occur as natural contaminant of poultry feeds (Edds and Bortell, 1983).

Aflatoxins are poisonous and cancer causing chemicals that are implicated in diseases such as liver cancer, genetic mutation, loss of weight, reduced feed intake and so on. The occurrence of aflatoxins in foods and feed is a problem of major concern all over the world. Profitability of poultry production can be greatly affected due to the frequency of feed contamination and the detrimental effects of these toxins on the performance of poultry birds (Hamilton, 1982). A major concern of aflatoxicosis is its interaction with protein, carbohydrate, lipid and vitamin metabolism. Aflatoxin also inhibits the synthesis of nucleic acids and proteins. It causes the depletion of glycogen by the impairment of the synthetic process in poultry. Aflatoxin B1 not only affects lipid synthesis and transport, but also interferes with its absorption and degradation in chicken (Aletor, 1991).

Oyegunwa *et al*., (2021) reported that some feed milling facilities in Ijebu Ode lack good storage facilities which make them have high incidence of contamination in maize, soybean meal, groundnut cake and wheat offal. Binder *et al*. (2007) found low concentrations of Deoxynivalenol, T-2 toxin and Zearalenone as contaminant in Europe (temperate areas). Elzupir *et al*., (2009) found a total of 64.29% animal feed (130.63ug/kg) and 87.50% manufactured animal rations (54.41-579.87 ug/kg) followed by 69.32% groundnut samples (4.07-79.85ug/kg) contaminated with AFs in Khartoum State of Sudan. Borutova *et al*. (2012) found a positive correlation between aflatoxin B1 and aflatoxin B2 prevalence on different feedstuffs i.e. corn, wheat, soybean meal, corn gluten meal, dried distiller grains, etc in Asia-Oceania region and concluded that the occurrence of single mycotoxin in any of the feedstuffs is rare.

Mycotoxins affect nutritional and economic value of staple foods such as maize, soy bean groundnut, wheat and so on especially in developing countries including Nigeria due to poor harvesting and storage conditions occasioned by climate change (Moss, 1991). In Nigeria, groundnut, maize and other cereals and legumes are sold in open market with less or no regulation of quality. For example, some of these raw materials when tested may contain above 12% moisture and this is a major factor that can subject such ingredients to attack by mycotoxins. Most of the contaminated feed raw materials find their ways into the major feed producing companies and popular feed millers in these countries without being checked. The resultant effect is the sale of feed that are of low quality to farmers and hence many aflatoxin related diseases are diagnosed in animals that consume such feed (Oyegunwa *et al*, 2015). While it has been found that aflatoxin affect a number of feed ingredients, not much information is available on the extent of prevalence of this mycotoxin in Nigerian feedstuffs.

The objective of this study therefore is to evaluate the levels of aflatoxin in four major poultry feed ingredients (maize, soybean meal, groundnut cake and wheat offal) purchased from four major feed mills in Ijebu Ode area of Ogun State of Nigeria.

Materials and methods

Experimental site

The field work of this experiment involving 4 major feed mills (A, B, C and D) was carried out in Ijebu Ode town, Ogun State of Nigeria lies between latitude 6^0N to 45^0N and longitude 3^0E to 57^0E in the South Western agro- ecological zone of Nigeria. Ijebu Ode is in the rain forest part of Nigeria with high relative humidity and high temperature. Laboratory analysis and aflatoxin quantification were carried out at the pathology laboratory of Animal Care Services Konsult, Ogere Remo, Ogun State of Nigeria.

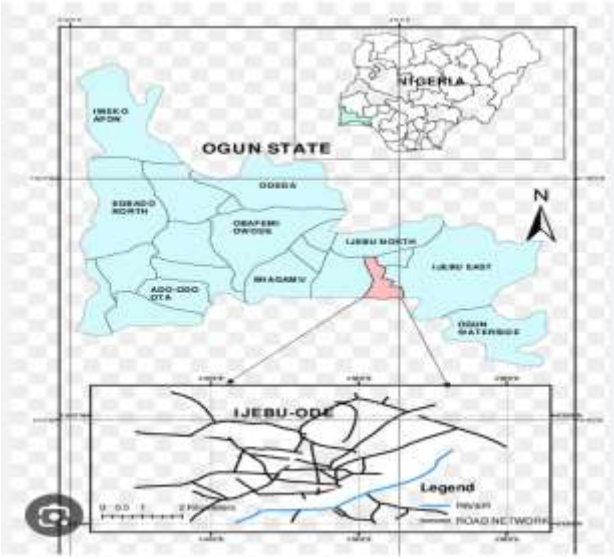

Figure 1: Map of Ogun State showing the study site.

Sampling of materials

The four feed materials used for this study (maize, soybean meal, groundnut cake and wheat offal) were randomly sourced from four selected major feed milling outlets in Ijebu Ode. The samples used were collected from different storage facilities of the feed mills and were thoroughly mixed in the bags before collection. The raw samples were replicated into 4 before analysis

Materials used for sample collection and aflatoxin estimation

Some of the materials used for sample collection and aflatoxin estimation include:

Sealable nylon for storing, Paper tape for labelling, hands gloves, electronic blenders, conical flasks, test tubes, filter papers, methanol, dilution ware, antibody coated well, burette, kits for aflatoxin and ELISA machine.

Samples collection and preparation

Sampling was done according to the method prescribed by Bainton *et al*. (1980) to give a representative sample. The representative samples (4 samples of each raw material) were later put in sealed bags and transported to the Poultry and Diseases Diagnosis and Aquaculture laboratory of Animal Care Services Konsult Ogere Remo, Ogun State.

The entire primary samples were ground to powder by milling and homogenized. Thereafter sub samples were made into different portions for aflatoxin level in the sample.

Procedure for extraction of aflatoxin

- ✓ 5grams of each feed ingredient is weighed into the conical flask
- ✓ 25ml of 70% methanol is poured to extract the aflatoxin.
- ✓ The resultant sample is filtered
- ✓ Pipette 900µl + 100ml of filtrate making a total of 1000µl
- ✓ Pipette 100µl of conjugate into dilution well
- ✓ Pipette 50µl from the diluets (900µl of 70% methanol + 100µl of filtrate)
- ✓ Pipette 100µl from dilution well into antibody coated well and timed for 15 minutes.
- ✓ Discard and was 3 times with distilled water
- ✓ Aflatoxin level was determined with ELISA reader at 450nm (nanometer). The ELISA machine read the colour and it gave the result in optical density to determine aflatoxin level. The deeper the colour the lower the aflatoxin level, the lighter the colour the higher the aflatoxin level.

Reading of the result on spectrophotometer

The optical density of a laboratory samples were read on the spectrophotometer to determine the actual concentration of aflatoxin in the samples. The values of the optical densities in each case is multiplied dilution factor to get the value of total aflatoxin in the samples.

Data analysis

The results obtained from this study were analysed using statistical analysis software (SAS, 1999) and means were compared with Duncan multiple range test.

Results and discussion

In all the feed ingredients tested, aflatoxins at different levels were discovered in them. Significant differences ($p< 0.05$) were observed in their aflatoxin levels across the 4 feed mills in Ijebu Ode (Table 1). From the table, the aflatoxin content of maize in feedmill A was the highest (25.93ppb) when compared with other feed mills. However, aflatoxin concentration in feed mill B, C & D were similar. Apart from feed mills B, C & D which had average aflatoxin B1 levels of 13.1, 15.28 and 15.28ppb respectively. Others were clearly above the 20ppb safe level (Coker *et al*., 1986). This pose a great danger for the farmers around Ijebu Ode for the fact that maize is the major energy supplying ingredients which forms more that 50% of the raw materials in poultry feed. An important factor that probably could be responsible for the high aflatoxin in maize in A is the available storage facility in the feed mill and the storage time, as our findings

further revealed that some of the samples have been in the poorly aerated/ventilated store for several months. Another reason could due to the fact that the study was carried out during the wet season. Most aflatoxin infection in maize occur in broken and damaged kernels and seed coat during the wet season (Vincelli *et al*., 1995).

For soybean meal, interestingly there were no similarities ($p<0.05$) in the concentration of aflatoxin in all the feed mills. However, the highest value of aflatoxin (157.85ppb) was recorded in feed mill D while the least value of aflatoxin (20.63ppb) was obtained in feed mill C. From the physical look at the soybean meal showed a darker colouration which may indicate possible heavy fungal infestation as a result of high moisture content.

Table 1: Average aflatoxin B1 levels of 4 major poultry feed ingredients from four major feed mills in Ijebu Ode, Ogun State Nigeria

Feed ingredients	Aflatoxin concentration (ppb)				
	Feed mill A	Feed mill B	Feed mill C	Feed mill D	SEM
Maize	25.93[a]	13.10[b]	15.28[b]	15.28[b]	1.39
Soybean meal	30.48[b]	24.78[c]	20.63[d]	157.85[a]	0.47
Groudnut cake	368.43[a]	289.28[b]	288.08[b]	157.45[c]	1.31
Wheat offal	36.63[d]	49.84[a]	26.88[d]	47.63[b]	0.56

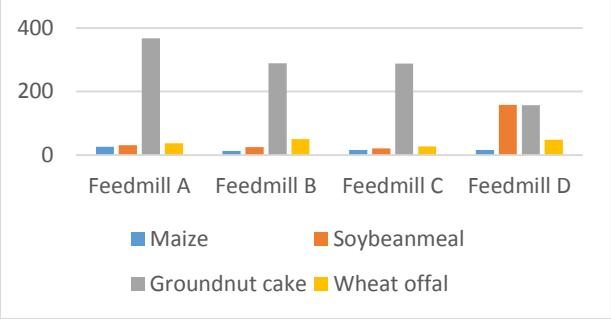

Figure 2: Level of contamination of feed ingredients with aflatoxin in the four major feedmills in Ijebu Ode

Aflatoxin is a major constraint to the use of groundnut in most producing countries worldwide (Okello *et al*., 2010). Economic yield losses may be up to 100% if the aflatoxin levels are beyond stipulated level. In table 1, the level of aflatoxin obtained in all feed mills sampled were significantly different. While feed mill A had the highest aflatoxin level for groundnut cake (368.43ppb), feed mill D recorded the lowest value (157.45ppb). Obviously from the result obtained for groundnut cake, high levels recorded may be due to high fungi infestation from the field or poor storage condition. Excessive moisture in field and in storage, temperature extremes, humidity, drought, variation in harvesting practices and insect infestations are a major environmental factors that determine the severity of mycotoxin contamination (Hussein and Brassel, 2001).
For wheat offal aflatoxin analysis, significant differences ($p<0.05$) were also observed in the values. The highest concentration of aflatoxin (49.84ppb) was obtained from feed mill B while the lowest (26.88ppb) was obtained from feed mill C. The same problem of storage condition and season of raw materials collection may be responsible for this high levels of aflatoxin contamination in poultry feed ingredients.

Conclusion and Recommendation

In conclusion, all the raw materials obtained from the four (4) feed mills in Ijebu Ode during the period of the research were contaminated with aflatoxin at different levels above the 20ppb safe limit. Only maize in feed mills B, C and D had aflatoxin values below 20ppb safe limit. Most of the feed mills lack good storage facilities and the grains were not properly dried before storage. This poses a great threat to livestock farmers around Ijebu Ode and environ. It is recommended that grains should be adequately dried and timely harvested before it is infested by fungi on the field. Good storage facilities should be put in place by the feed miller while animal feed regulatory body should inspect the feed mill facilities before granting operational licences to them. Occasional drying and turning by local farmers can also be practiced.

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