

## **Influence of climate change on Aflatoxin levels of some poultry feeds collected from feed mills in South-Western Nigeria.**

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### **Abstract**

Study on the aflatoxin contaminating poultry feeds in South-Western zone of Nigeria was carried out in May- October 2008 and November 2008-April 2009 which represent two seasons of the year during the rainy and Dry season. A total of 300 samples of finished commercial poultry feeds (broilers and layers mash) collected from different feed mills in South-Western (Lagos, Ogun, Oyo, Osun, Ondo and Ekiti State) Nigeria and were examined for aflatoxin contamination. Extraction of aflatoxin from feed samples was carried out both by the aqueous acetone and the chloroform extraction method. The extracts were qualitatively examined by quick screening method and thin layer chromatography method. Aflatoxin B1 in the feed samples were detected in the range of trace 10.5  $\mu\text{g kg}^{-1}$  and 65.3  $\mu\text{g kg}^{-1}$ . Aflatoxins B2, G1 & G2 were also present in addition to aflatoxin B1. Reduction was observed in aflatoxin B1 content of the broilers feed mash during dry season as compared to the rainy season. The lowest aflatoxin B1 concentrations were recorded for samples collected from Ekiti State while Lagos State had the highest mean concentration in May- October 2008 and November 2008-April 2009 period respectively the results also show that the locations where the samples were collected had a significant effect on

aflatoxin B1 levels. The lowest aflatoxin B2 concentrations were recorded for samples collected from Ekiti state while Lagos State had the highest mean concentration in May- October 2008 during rainy season and November2008-April 2009 period dry season respectively. There was reduction in the mean concentration of aflatoxin B2 during dry season in the samples collected from all the state. Reduction in G1 concentration was observed throughout the season of November2008-April 2009 period (dry season). Aflatoxin G2 was not detected from the broiler mash collected from Ekiti and Ondo state The highest aflatoxin G2 concentrations was recorded for samples collected from Oyo state in May- October 2008 and Lagos State in November 2008-April 2009 period respectively. The least mean concentration was observed in Lagos State in in May- October 2008 period. The least mean concentration of aflatoxin B1 of layers mash was recorded for samples collected from Ekiti state while Osun State had the highest mean concentration during the rainy season in May- October 2008 period. The highest concentration was observed from samples collected from Ogun State during the dry season in November2008-April 2009 period. The result reveal that aflatoxin contamination of feed is more abundant during rainy season period in South-West Nigeria than dry period therefore, further monitoring of aflatoxin level of feeds in the country should be frequently carried out.

**Key words:** climate change, poultry feeds, mycotoxin, South-Western, Nigeria.

## Introduction

Feeds supply livestock with nutrients necessary for their body functions and are therefore indispensable for livestock production. Feed quality has been specified on the basis of nutritional value of every individual feed component. However, any given natural feed material also contains various non-nutritional contaminants that may reduce its nutritional value or even exert adverse health effects in animals (Fink-Gremmels, 2004). Many parasitic and saprophytic fungi infect growing crops and may continue to develop through post-harvest, processing and formulation of finished feeds (Vieira, 2003; Mabbett, 2004). Fungal growths cause direct losses in volume and quality of feed ingredients and subsequently feeds made from them, leaving behind some poisonous mycotoxins, which contaminate feed raw materials and finished feeds. Moulds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrient in the feed. Such contamination is widespread, especially in tropical countries where poultry production and processing are expanding rapidly (Mabbett, 2004). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins, trichothecenes such as DON and T-2, ochratoxin and some fusariotoxins (Mabbett, 2004, Shepherd 2008). Ingestion of higher concentration however leads to acute clinical symptoms associated with specific vital organs, the immune system and other aspects of avian physiology as well as mortality (Mabbett, 2004). According to Richardson (1995), quality control in the feed industry is usually the responsibility of management. This includes documentation and policing of various procedures and processes necessary to guarantee the basic quality of feedstuff and feeds. Commercial feeds manufacturers in developing countries like Nigeria may however not conform to this (Omede, 2004). Generally, mixed feeds by self-composition, especially under favorable conditions, such as high moisture and increased

temperature represent excellent substrate for growth and reproduction of fungi. Fungal and mycotoxin contamination of feedstuff and mycotoxicoses farm animals however, remain neglected in livestock production research issues in Nigeria (Okoli, 2005).

Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds. They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world (Vieira, (2003). The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods. Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans. (Okoli, 2005).

Aflatoxins often occur in crops in the field prior to harvest. Postharvest contamination can occur if crop drying is delayed and during storage of the crop if water is allowed to exceed critical values for the mold growth. Insect or rodent infestations facilitate mold invasion of some stored commodities. (Philip 2004):

### **Factors Favorizing Aflatoxin Production**

Fungal growth and aflatoxin contamination are the consequence of interactions among the fungus, the host and the environment. The appropriate combination of these factors determine the infestation and colonization of the substrate, and the type and amount of aflatoxin produced (Reddy and Waliy 2007). However, a suitable substrate is required for fungal growth and subsequent toxin production, although the precise factor(s) that initiates toxin formation is not

well understood. High water, high-temperature stress, and insect damage of the host plant are major determining factors in mold infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities, and weed competition have been associated with increased mold growth and toxin production. Aflatoxin formation is also affected by associated growth of other molds or microbes

### **Safe level for Aflatoxin:**

According to experts, there is no safe level and risk depends on the amount of aflatoxins in the feed and also on presence of other mycotoxins in the feed. The Food and Drug Administration (FDA) of United States has set a maximum allowable level of aflatoxins at 20 ppb. The safe level for aflatoxins vary from one form to another form due to variation in managerial conditions and disease prevalence.

Following harvest and during shipment and storage of agricultural commodities, toxigenic mould growth and potential mycotoxin production are influenced by many factors including moisture level, temperature, aeration, infestation by insects and other microorganisms, storage time, chemical treatments, spore infection density and storage conditions (especially leakage of water or condensation) (Philip, 2004)

There is therefore an urgent need to understand the impact of mycotoxin in animal production in Nigeria. Strategies for reduction of mycotoxin contamination in animal production in Nigeria should however begin with a clear understanding of fungal organisms involved and the type of toxins they produce (Okoli, 2005, Opara and Okoli, 2005). Such information could be utilized in selecting appropriate mould inhibitors and mycotoxin binders for the feed industry in Nigeria. The objective of this study is to determine the influence of seasonal variation on the level of

aflatoxin in feeds collected from South-Western Nigeria during the rainy and dry season months of May- October 2008 and November 2008-April 2009 period.

## **Materials and methods**

### **Samples collection and preparation**

A total of 300 samples of feed mixtures, designed for poultry feeding, were obtained from different feed mills in six states (Lagos, Ogun, Oyo, Osun, Ondo and Ekiti State) in South-Western Nigeria in the period of May-October 2008 and November-April 2009 periods which represent Rainy and Dry seasons of the year. Twenty five feeds samples of broilers and layers mash respectively were collected from feed mills in a sterile paper bag in each state. The samples represented the following two poultry feed categories: chicken broilers-growing mash (150 samples) and chicken layers mash (150 samples).

### **Aflatoxin Analysis of the feed samples**

The extraction method of (AOAC 2000, Reddy and Hayes 2001, Scudamore; *et al* 1998 and Adegoke, 2004) was modified as follows,

20 g sample of dry blended feed sample was weighed into a conical flask, 80 ml of 85 % methanol (methanol) water 85:15 was added and blended for 3mins in warring commercial lab blender.

The extract was shaken for 30mins at 7000rpm and later filtered though what man filter paper No 1 and 40% of the filtrate was transferred into 250ml separating funnel and 40ml of 10% sodium chloride was added and shaken. Thereafter, 25ml of normal hexane was added and shaken for 1min and the mixture was allowed to separate into two layers with the methanol and hexane on top and aflatoxin and 10% NaCl (40ml) at the bottom The lower portion was drained into a conical flask

containing 25ml of chloroform and pour back into the separating funnel and was vortexed for 1min, it was drained and separated into flask and allow to evaporate to dryness under fume hood.

The dry extract was dissolved in 1ml chloroform. Four (4ml) of the dissolved extract was spotted on thin layer chromatography (TLC) using 20 x 20 cm plates coated with silica gel (0.5 mm thickness) and developed in a tank containing 90% chloroform, 10% acetone and 1% isopropanol and the developed plate was observed under ultraviolet light at 366nm wave length to separate the toxigenic isolate from the intensity of the spot to those of the standard.

Aflatoxin quantification was done by scanning the TLC plates in a CAMAG TLC Scanner 3 (densitometer), which measured the absorbance and fluorescence

## Results

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### Levels of aflatoxin in the broilers mash feed in South-Western zone Nigeria

The levels of aflatoxin B1 ( $\mu\text{g kg}^{-1}$ ) in the broilers mash investigated in the zones are presented in Fig1 Reduction was observed in aflatoxin B1 content of the broilers feed mash during dry season ( $10.5 \pm 0.01^a - 30.5 \pm 0.01^j \mu\text{g kg}^{-1}$ ) as compared to the rainy season ( $18.50.01^d - 45.6 \mu\text{g kg}^{-1}$ ). The lowest aflatoxin B1 concentrations were recorded for samples collected from Ekiti state ( $18.5 \pm 0.01^d, 10.5 \pm 0.01^a \mu\text{g kg}^{-1}$ ) while Lagos State had the highest mean concentration ( $45.6 \pm 0.01^p, 30.5 \pm 0.01^j \mu\text{g kg}^{-1}$ ) in May- October 2008 and November 2008-April 2009 period respectively the results also show that the locations where the samples were collected had a significant effect on aflatoxin B1 levels.

The lowest aflatoxin B2 concentrations were recorded for samples collected from Ekiti state ( $18.5 \pm 0.01^d$ ,  $12.7 \pm 0.01^b$   $\mu\text{g kg}^{-1}$ ) while Lagos State had the highest mean concentration ( $45.6 \pm 0.01^p$ ,  $35.8 \pm 0.01^l$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period during rainy season and November 2008-April 2009 dry season respectively. There was reduction in the mean concentration of aflatoxin B2 during dry season in the samples collected from all the state as shown in Fig 2.

The result of aflatoxin G1 and G2 concentration of the broilers feed samples as shown in Fig 3 and 4 Indicated that feed samples collected from Ekiti State had the lowest aflatoxin G1 concentrations ( $25.3 \pm 0.01^g$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period while Oyo State had the least mean concentration ( $18.5 \pm 0.01^d$   $\mu\text{g kg}^{-1}$ ) in November 2008-April 2009 period. Ondo State had the highest mean concentration ( $83.2 \pm 0.01^v$   $\mu\text{g kg}^{-1}$ ), followed by Lagos State ( $75.3 \pm 0.01^u$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period. Reduction in G1 concentration was observed throughout the season of November 2008-April 2009 period (dry season). Aflatoxin G2 was not detected from the broiler mash collected from Ekiti and Ondo state as Shown in Fig 4. The highest aflatoxin G2 concentrations was recorded for samples collected from Oyo state ( $43.2 \pm 0.01^o$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 and Lagos State period ( $21.73 \pm 0.01^e$   $\mu\text{g kg}^{-1}$ ) in November 2008-April 2009 period respectively. The least mean concentration was observed in Lagos State in ( $25.53 \pm 0.01^g$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period.

### **Levels of aflatoxin concentrations in Layer's Mash feeds collected from South- Western zone Nigeria**

Aflatoxin B1 concentrations in the feed samples analyzed ranged from  $10.5 \pm 0.01^a$   $\mu\text{g kg}^{-1}$  and  $63.3 \pm 0.01^s$   $\mu\text{g kg}^{-1}$ . The levels of aflatoxin B1 ( $\mu\text{g kg}^{-1}$ ) in the layers mash investigated in the zones are presented in Fig 5 The least mean concentration of aflatoxin B1 was recorded for



samples collected from Ekiti state ( $29.53 \pm 0.01^i$   $\mu\text{g kg}^{-1}$ ) while Osun State had the highest mean concentration ( $63.6 \pm 0.01^s$   $\mu\text{g kg}^{-1}$ ) during the raining season in May- October 2008 period. The highest concentration was observed from samples collected from Ogun State ( $42.5 \mu\text{g kg}^{-1}$ ) during the dry season in November 2008-April 2009 period.

Aflatoxin B2 concentrations in the feed samples analyzed ranged from  $10.5 \pm 0.01^a$   $\mu\text{g kg}^{-1}$  and  $53.6 \pm 0.01^q$   $\mu\text{g kg}^{-1}$  during the rainy season in May- October 2008 period. The result in Fig 6 showed that Osun State has the highest B2 concentration ( $53.6 \pm 0.01^q$   $\mu\text{g kg}^{-1}$ ) and the least was recorded from Ogun State ( $36.25 \pm 0.01^m$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period while Lagos State had the highest B2 concentration ( $38.7 \pm 0.01^n$   $\mu\text{g kg}^{-1}$ ) in the dry season.

The result of the mean concentration of aflatoxin G1 of the poultry feeds are shown in Fig 7. The highest concentration of aflatoxin G1 was observed in feeds samples collected from Lagos State) during rainy and dry season respectively ( $54.5 \pm 0.01^r$   $\mu\text{g kg}^{-1}$  and  $68.7 \pm 0.01^t$   $\mu\text{g kg}^{-1}$ ) while the least concentration was observed in the samples collected from Ondo and Osun State ( $22.6 \pm 0.01^f$   $\mu\text{g kg}^{-1}$  and  $35.5 \pm 0.01^l$   $\mu\text{g kg}^{-1}$ ) during the rainy season of May- October 2008 period.

The result of aflatoxin G2 concentration of the broilers feed samples as shown in Fig 8. Aflatoxin G2 was not detected in the feed samples collected from Ekiti and Ondo State in the two seasons of the year. While Feed samples collected from Oyo State had the highest aflatoxin G2 concentrations ( $32.5 \pm 0.01_k$   $\mu\text{g kg}^{-1}$ ) in November 2008-April 2009 period (dry season) and Lagos State had the least mean concentration ( $15.7 \pm 0.01^c$   $\mu\text{g kg}^{-1}$ ) in the same season. Ogun State had the highest mean concentration ( $28.5 \pm 0.01^h$   $\mu\text{g kg}^{-1}$ ) in May- October 2008 period.

## **Discussion**

Assessment of the incidence of aflatoxin contamination of some feeds collected from south-Western Nigeria as conducted in this work gives the quality of the studied feed products.

Aflatoxin in the feed samples were analyzed using thin-layer chromatographic technique which is perceived by modern scientists as an insensitive technique but to the scientists in the developing world, it remains a simple, quick and inexpensive procedure for mycotoxin analysis.

The toxin was found as a contaminant of poultry feeds all year round with a higher incidence during the rainy season than the dry season.

The detection limit of the technique as determined in the present study as well as by other workers (Bankole and Mabekoje, 2004) with regards to aflatoxins particularly B1 is 10.5 µg/kg which is quite sensitive enough to meet the standards of both national and international regulatory agencies and therefore validates the results obtained. Aflatoxin B1 contamination of feed is a worldwide problem and Nigeria is not an exception as this study has shown contamination of marketed feeds in the south-western Nigeria. Garrido et al. (2003) detected the toxin in 79.9% of milk samples collected from Brazil, at concentrations between 0.05 and 0.2 µg/kg. The hazards of aflatoxin B1 have been fully recognized; of great public health concern is the fact that the toxin is a potent carcinogen. Based on the result obtained. (Odoemelam and Osu, 2009) in Nigeria and elsewhere. Fungal growth and mycotoxin contamination are dependent on climate and storage conditions and therefore vary with locations, with hot and humid climate, poor storage conditions and poor agricultural practices exacerbating fungal and mycotoxin

contents in foods and feedstuffs . Grains in rural area of South-Western Nigeria which is where our samples are from, are handpicked at harvest, left to dry for weeks at a threshing ground, threshed, packaged and transported in sacks to markets where they are sold in open containers. The complex effects of relative humidity, temperature, precipitation and insect and rodent infestation and their daily variation may interplay to provide conditions conducive for fungal growth and aflatoxin contamination on such exposed and poorly stored grains. These methods of harvesting, storage and transportation which differ from those in other parts of the country could probably explain the more elevated incidence and contents of aflatoxin contaminations in the feeds in this work as compared to results of the other workers (FAO/WHO, 2004).

The significant effect of locations on the incidence and aflatoxin levels of the feed samples implies that the samples were from sources with different relative humidity, temperature, storage conditions and even agricultural practices.

The humid conditions of the South- Western zone of Nigeria during raining (May –October) seasons are more favorable for fungal growth and mycotoxins production than the cold drier climate (Nov-April) hence the higher toxin contamination in the former than the later.

Aflatoxin concentrations in the feed samples analyzed were above the National Agency for Food and Drug Administration and Control (NAFDAC) and European Union (20 ug/kg) tolerance level for aflatoxin in grains for animal consumption Marasas WFO (2001).

The chronic consumption of these feeds with unsafe levels of AFB<sub>1</sub>, that is, immunosuppressive, nephrotoxic and hepatocarcinogenic Bhat and Vasanthi (2003) has grievous public health implications which calls for control and regulation of mycotoxins in the country. The desired control of mycotoxins can be achieved by reducing fungal infection of crops by rapid drying and correct storage of the harvested crops using effective anti-mould preservatives. Properly

designed, mycoflora and mycotoxin surveys and monitoring programmes can reduce the fungal and mycotoxin in our foods and feeds.

The induction of cancer by aflatoxins has been extensively studied. Aflatoxin B<sub>1</sub>, aflatoxin M<sub>1</sub>, and aflatoxin G<sub>1</sub> have been shown to cause various types of cancer in different animal species (Fink-Grammels 2004). However, only aflatoxin B<sub>1</sub> is considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals to be identified as a carcinogen.

Aflatoxin contamination in food and feed supplies may have serious consequences for human and animal health. Aflatoxins are commonly associated with groundnuts, dried fruit, tree nuts, spices and cereals. Contamination is most acute and widespread in the warm, humid areas of Africa, Asia and Latin America. However, aflatoxins are found in temperate areas of North America and Europe as well. Contamination can occur in crops in the field, at harvest, during postharvest operations and in storage (Mabbett 2004). The rate and degree of aflatoxin contamination are dependent on temperature, humidity and soil and storage conditions (Mabbett 2004). This result reveals that most feed samples were from unhygienic storage condition. This finding is similar to the finding of Mabbett, (2004) who reported mycotoxigenic fungi in some feed samples. The need for setting maximum levels of aflatoxins in foods and feeds is generally recognized. Several countries, particularly some industrialized ones, have already set specific limits, ranging from 0 to 30 µg/kg for aflatoxin B<sub>1</sub> in foodstuffs and from 0 to 50 µg/kg for total aflatoxins.

The setting of internationally agreed maximum tolerable levels for aflatoxins in food and feed is of global importance. Recently, 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  $\mu\text{g}/\text{kg}$  and 20  $\mu\text{g}/\text{kg}$  for aflatoxin B<sub>1</sub> in food. Unnecessarily low guideline levels for aflatoxin may result in a technical barrier to trade (FAO.2004).

These findings provided evidence that there are aflatoxin problems in these localities. The analysis of natural occurrence of aflatoxin in poultry feed samples further substantiated the probability of aflatoxin ingestion by the poultry birds. The high incidence of naturally produced aflatoxin in the samples during the rainy season illustrates the hazards with which these communities are confronted.

### **Conclusion**

In view of the ubiquitous occurrence of potential toxin producers in all sampling regions, further monitoring of aflatoxin in feeds from the relatively hot, humid western and southwestern regions of the country, coupled with detailed analysis of preharvest and storage factors is justified.

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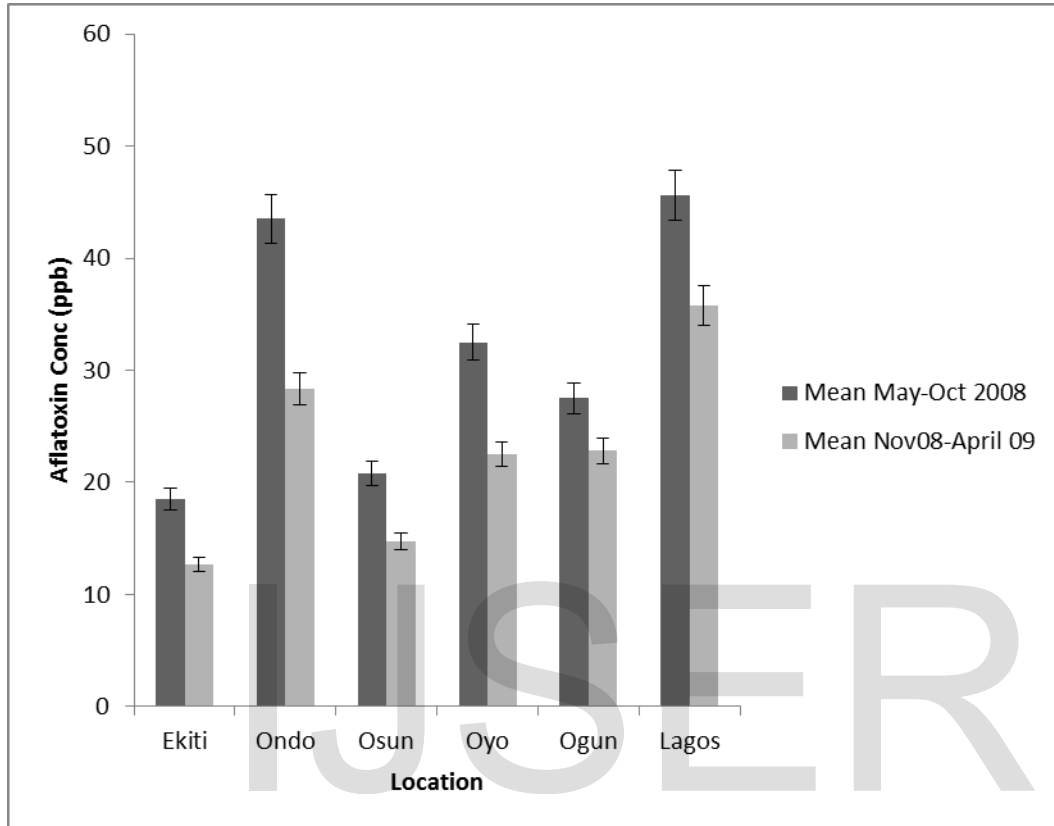
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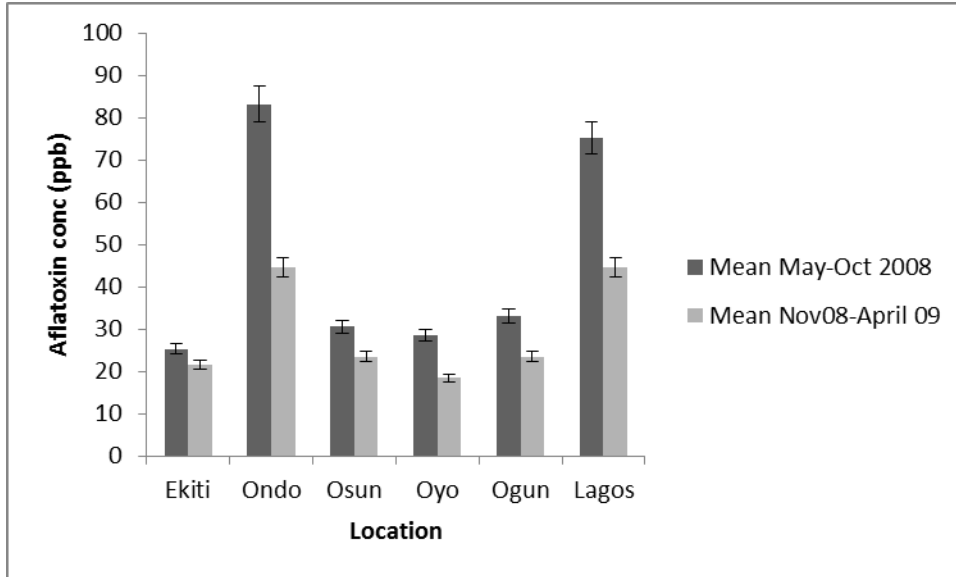
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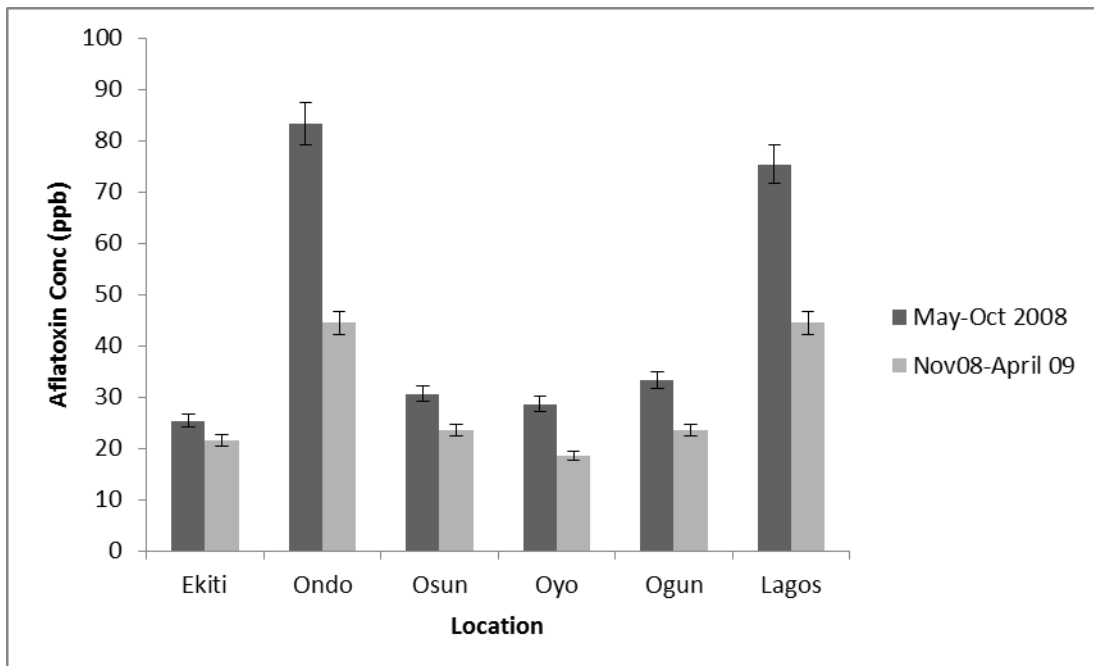


**Fig 1: Levels of aflatoxin B1 (ppb) in broiler's Mash collected in Western zone of Nigeria**

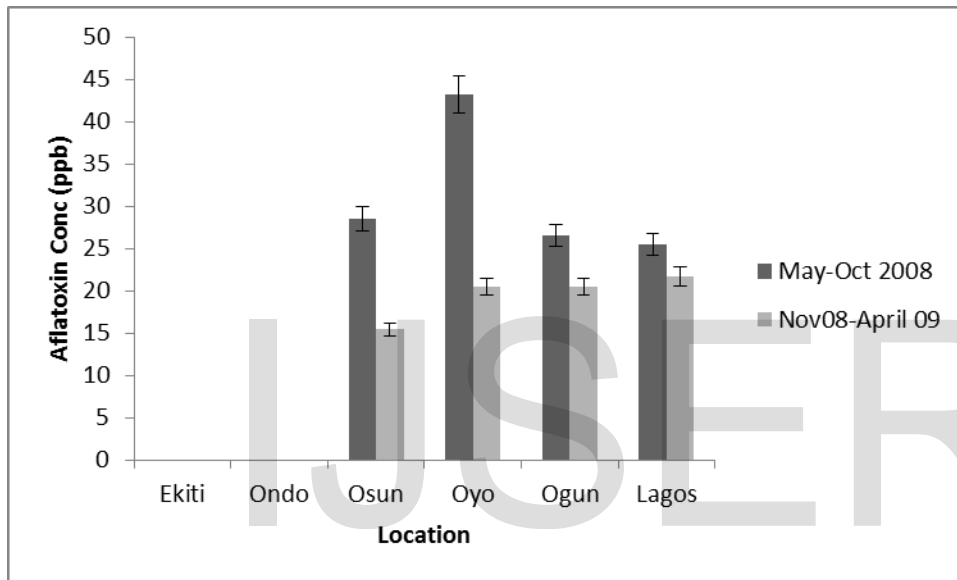


**Fig 2: Levels of aflatoxin B2(ppb) in broiler's Mash collected in Western zone of Nigeria**

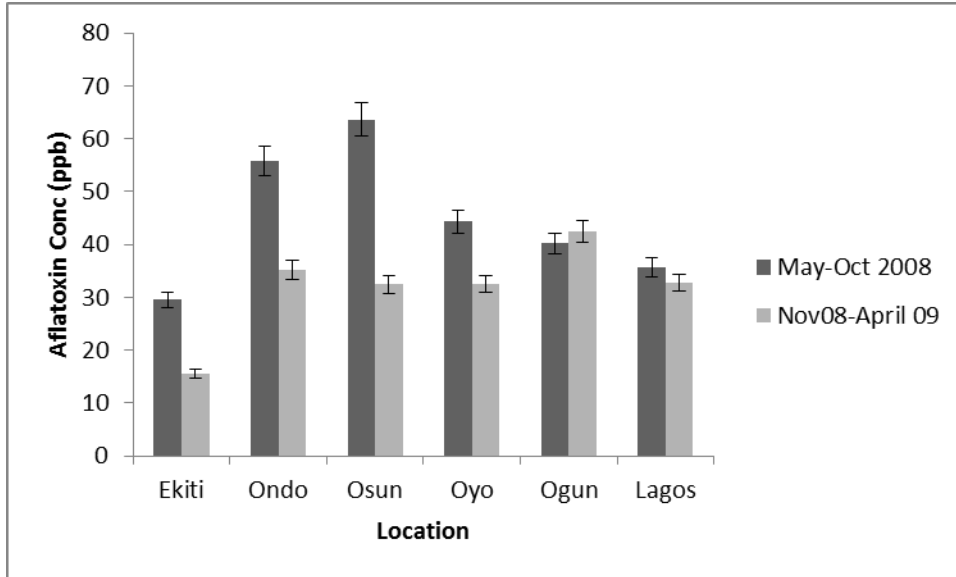
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**Fig 3: Levels of aflatoxin G1 (ppb) in broiler's Mash collected in Western zone in Nigeria**

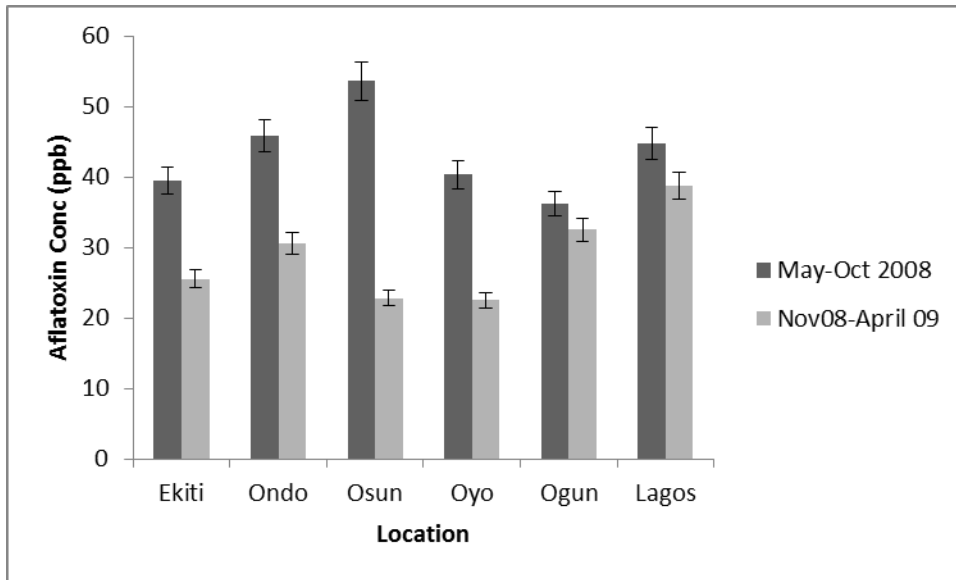


**Fig 4: Levels of aflatoxin G2 (ppb) in broiler's Mash collected in Western zone in Nigeria**

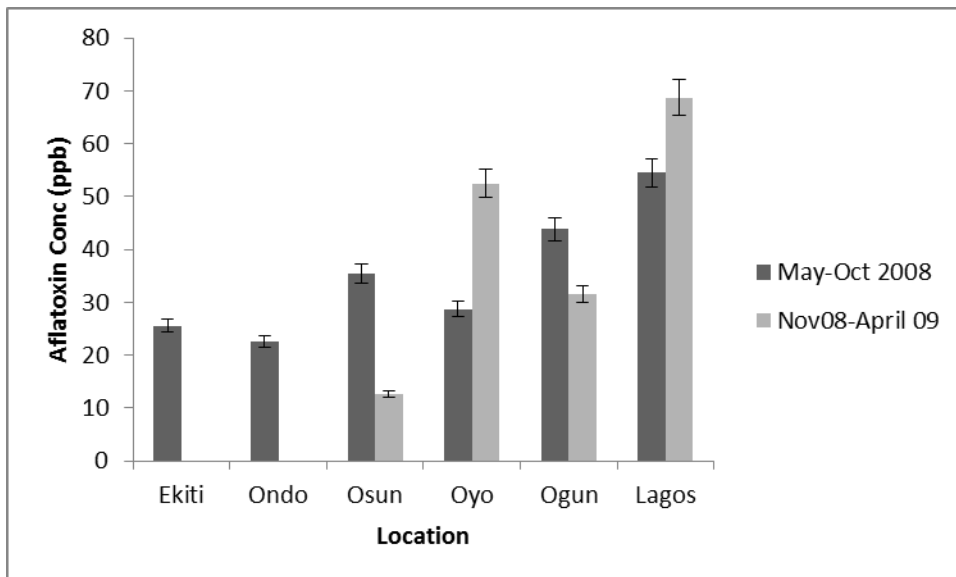


**Fig 5: Levels of aflatoxin B1(ppb) in Layers Mash collected in Western zone in Nigeria**

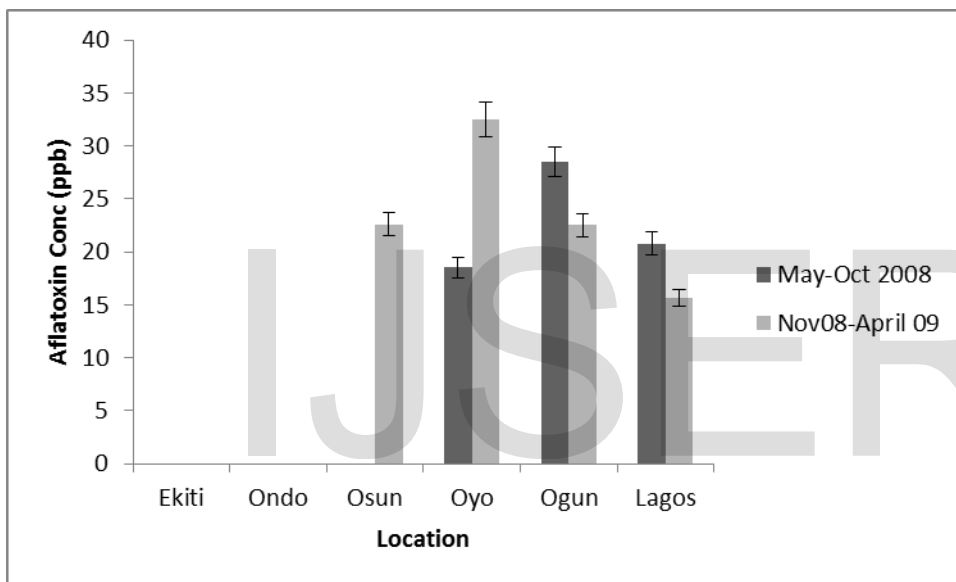
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**Fig 6: Levels of aflatoxin B2(ppb) in Layers Mash collected in Western zone in Nigeria**



**Fig 7: Levels of aflatoxin G1 (ppb) in Layers Mash collected in Western zone in Nigeria**



**Fig 8: Levels of aflatoxin G2 (ppb) in Layers Mash collected in Western zone in Nigeria**