

The impacts of seasonal variation of Aflatoxins load on some pigs feeds in the tropics of Niger delta

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ABSTRACT

The research was conducted to study the impacts of seasons on aflatoxins loads of some pig's feedstuffs in the humid tropics was investigated, the feedstuffs sampled were cassava peels, cocoyam peels and palm kernel cake. The study lasted for thirty-two weeks. These feedstuffs were collected in two piggery farms in each of the four LGAs that make up Uyo zone of Akwa Ibom state, in both dry and rainy seasons. The collected feedstuff samples were analyzed in the laboratory for aflatoxins concentration levels using AOAC method of thin-layer chromatography (TLC) with some modifications. Data were collected from the aflatoxins laboratory analysis of these feedstuffs. The results of this study indicated the presence of aflatoxins in all the sampled feedstuffs, however the lowest concentration of aflatoxins which is 0.011ppb is obtained in cocoyam peels and cassava peels while the highest concentration of aflatoxins obtained is 0.055 ppb in palm kernel cake, these values are within the tolerance limit in Nigeria for total aflatoxins, which is 20 ppb. The obtained aflatoxins levels are within range and poses less risk to pigs and pork consumers. The results also shows that there were no significant difference ($P > 0.05$) on the aflatoxins concentration levels of the feedstuffs at their sources, across the piggery farm locations, in the season and source of feeds interactions, as well as season and feedstuffs interactions. However the main effect of season on aflatoxins level which is 0.022 ppb and 0.044 ppb in the dry and rainy season respectively were highly significant ($P < 0.01$). Also the main effect of aflatoxins of the feedstuffs were highly significant ($P < 0.01$). There were higher values in the aflatoxins levels in all the feedstuffs sampled in the rainy season than in the dry season. It was therefore concluded that season has an impacts on the aflatoxins level of pig's feedstuffs with rainy season favouring toxigenic mould metabolism and growth in the feedstuffs which produces aflatoxins as their metabolites than in the dry season in this study, hence adequate preservative measures, proper feed handling and storage should be adopted. It is therefore recommended that piggery farmers should adopt adequate preventive measures and also apply the various preservative storage methods in order to reduce the aflatoxins levels in feedstuffs especially in the rainy season for better performance of the pigs and safety of pork consumers.

Keywords: Aflatoxins, Feedstuff, Humid tropics, Pigs.

1. INTRODUCTION

The high cost of feed remains the greatest challenge to livestock production particularly monogastric animals in Nigeria. Grains which form bulk of concentrate feed are in short supply and expensive. Moreover, the price of animal protein concentrate has risen astronomically. In Nigeria, cereal grains are in high demand and its production has never been adequate to meet the needs of the increasing population; consequently there is little or no excess grains for livestock feeding. Adesehinwa *et al.* [1-2] stated that the use of alternative feedstuffs such as cassava peels, brewer spent grains, palm kernel cake and bambara nut waste in feeding pigs is a way of reducing the cost of pork production. These feedstuffs are procured and stored for a given period of time with different handling and storage techniques before feeding them to the pigs. Within this period of storage and handling, the feedstuffs may be exposed to mould infestation and the resultant aflatoxins contamination [3]. Henry (2009) [4] stated that poor handling and storage of feedstuffs adversely affect the quality and the nutrients supplied by the feedstuffs to the animals [5]. Pig farmers may be feeding their pigs with mould contaminated feedstuffs without knowing that such feedstuffs are contaminated.

Aflatoxicosis in pigs reduces feed intake, reduce weight gain, reduce growth rate, immunosuppressive including antibody and interleukin production, causes liver damage and hemorrhage [6, 7, 8, 9]. Earlier reports [2, 10] state that a major solution to the problem of rising costs, scarcity of energy and protein sources for monogastrics is to seek for new and non-conventional feed resources which are able to replace a certain percentage proportion of the known conventional feedstuffs without any deleterious effect on the animal performance. Esonu *et al.* (2001) [11] indicated that such substitute which

have been successfully used to replace the conventional feedstuffs are brewer spent grains, stem/tuber residues and cereal by-products such as wheat offal, rice bran, maize bran as well as bambara nut waste and cassava or yam peels.

Onyimonyi and Ugwu [2, 12] had studied the feeding value of such non-conventional feed resources as palm kernel cake, bambara nut offal, rice husk and cassava peels, and that they can be utilized in supplementing a given portion of the conventional feedstuffs. Onyimonyi and Ugwu (2007) [12] further noted that these non-conventional feeding stuffs are commonly found in the South Eastern Nigeria. These alternative feedstuffs have proved valuable in supporting the performance of livestock and poultry and that by utilizing them in ration formulation, it will go a long way in reducing the demand and cost of conventional feedstuffs, which greatly increases the total cost of pork production [13]. Pigs are known to be highly prolific and very efficient in converting feed nutrients into high quality animal protein [14]. Adesehinwa (2008) [1] stated that pigs are used to convert a variety of feeds into meat for human consumption. The supply of animal protein by pigs is limited by many factors among which are inadequate nutrition and feeding; poor breeding stocks, poor management practices and housing; disease prevention and control in pig production [15, 2].

Pitt and Miscamble [16] defined mycotoxins as toxic secondary metabolites produced by micro-organisms of the fungus kingdom commonly known as moulds. Some studies reported the ability of moulds to produce toxins potentially dangerous to man and animals are common and widespread [17]. Odoemela and Osu, (2008) [18] reported that toxigenic moulds have been found during growth, harvest and storage of different foods and feeds and other agricultural produce. There are five major agriculturally

important fungal toxins of economic importance namely; aflatoxins, deoxynivalenol, ochratoxin, zearalenone and fumonisin, which are produced by fungi invasion of agricultural produces and feed ingredients under favourable conditions of temperature (25 - 30°C) and relative humidity of above 80% [19-20]. The moulds contaminate a large number of dietary staples and agricultural produce such as rice, corn, cassava, peanuts and spices, while noting that humans and animals are exposed to aflatoxins by consuming contaminated foods and feeds [21]. These fungal metabolites which when ingested, inhaled or absorbed through the skin causes lowered performance, sickness or eventually death in both animals and humans [22, 23].

Several moulds and yeast species were isolated from grains with *Fusarium verticilloides* and *Aspergillus flavus* having the highest frequency of occurrence of 39.1% and 22.3%, respectively [24]. Moulds have the potentials of forming aflatoxins and trichothecenes in grains, peanuts, cottonseed and other agricultural products [24]. The *Aspergillus* spp. produces aflatoxins [25]. The high incidence of mycotoxins contamination could be due to physical status of grains, the moisture content, temperature, oxygen and the amount of carbon (iv) oxide in the atmosphere [26]. These factors are reported to influence the rate of infestation and proliferation of moulds in agricultural produce and grains, especially under storage condition.

In respect to climate, two studies by [27], suggest that aflatoxins are expected to spread and become more problematic with future climate conditions [18, 24]. There are many climatic reasons for an increase in aflatoxins, including an increase in temperature, humidity, moisture and rainfall [27].

Studies suggested that due to a rise in extreme temperatures, many countries in these regions that

are currently susceptible to drought, such as sub-Saharan Africa, Asia and Latin America, may actually see a decrease in aflatoxins due to lower crop yields [27, 28]. Also, an increase in temperature could lead to an increase in plant stress and an increase in the number of plants that are more susceptible to disease, which might suggest that the crops that are grown in developing countries are even more prone to containing aflatoxin-producing fungus. The aflatoxins are likely to occur in field stressed crops than the unstressed crops [29].

Climate change may also influence the impact of aflatoxins contamination in temperate climates. In the studies [27], they stated that the two climate variables that are important predictors of aflatoxins are warm night temperatures and precipitation immediately before or during the harvest. Precipitation prior to a harvest increases the water content in crops, which means that it is much more difficult to keep crops dry or free of fungal growth. Further, warm night temperatures allows for greater insect activity, which could increase the spread of fungus. As such this study is aimed at investigating the impacts of seasonal variation of aflatoxins loads on some pig's feeds in the tropics in Niger Delta.

2. MATERIALS AND METHODS

2.1. Location of study

The experiment was conducted at the Swine unit of the Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State. Akwa Ibom state is in Nigeria. It is located in the coastal southern part of the country, lying between latitudes 4°32'N and 5°33'N, and longitudes 7°25'E and 8°25'E. The state is located in the south-south geographical zone, and is bordered on the east by Cross River State, on the west by Abia State, and on the south by Atlantic Ocean and the southernmost tip of Cross Rivers State.

Table 1. Effect of source of feedstuffs on the Aflatoxins levels of pig feedstuffs

Feedstuffs	Source	Aflatoxin level (ppb)	P-value
Cassava peels	Itu	0.032±0.003	0.157 ^{NS}
	Ibiono	0.042±0.002	
Cocoyam peels	Ibesikpo	0.031±0.001	0.522 ^{NS}
	Itam Mkt.	0.033±0.003	
Palm kernel cake	Akpan oil mills	0.034±0.002	0.283 ^{NS}
	Johnson oil mills	0.031±0.003	

Units = (ppb): parts per billion, NS = not significant.

The climate of the study area is tropical with relative humidity ranging from 60 to 90 %. The average diurnal minimum temperature ranges from 22oC to 24.7oC while the average maximum temperature ranges from 33oC to 37oC [30]. The annual rainfall ranges from 1680 to 1700 mm [31], while the natural day length for Uyo is between 12 and 13 hours [30]. The rainy season is between April and October and the dry season is between November and March Feedstuff samples were collected at their major sources and from those stored in the piggery farms for pig feeding, both in the dry and rainy season; mainly in December to January for the dry season and June to July for the rainy season, this is to evaluate the effect of seasons on aflatoxins level i.e. mould contamination in the various feedstuffs used in feeding pigs by piggery farmers. The study lasted for thirty two weeks from December, 2019 - July, 2020.

2.2. Experimental Materials

The experimental materials were the feedstuffs used by pig farmers in feeding pigs in Uyo zone. Some of the major feedstuffs used to feed pigs in the piggery farms are cassava peels, cocoyam peels and palm kernel cake. Others include yam and potatoes peels, plantain leaves and its peels, food remnants and crop residues. These materials were gotten around the local government areas around Uyo zone Akwa Ibom State. These are the major sources of these feedstuffs from where the piggery farmers buy their feedstuffs directly or indirectly from local traders.

2.3. Experimental Procedure

Four pig farms were randomly selected in four local government areas in Uyo zone which are Itu, Ibesikpo, Ibiono and Uran. Feedstuff samples were collected from their major sources from where the

Table 2. Effect of farm location on Aflatoxins levels of pigs feedstuffs

Feedstuffs	Location (Piggery farms)				P-value
	A	B	C	D	
Cassava peels	0.048	0.041	0.036	0.032	0.424 ^{NS}
Cocoyam peels	0.033	0.034	0.034	0.03	0.532 ^{NS}
Palm kernel cake	0.034	0.029	0.029	0.032	0.4005 ^{NS}

NB: A = Itu; B = Ibesikpo; C = Ibiono; D = Uruan local government areas of Akwa Ibom State

Table 3. Main effect of season on Aflatoxins levels of pig feedstuffs

	Season		SEM	P-value
	Dry	Rainy		
Aflatoxins levels	0.036 ^a	0.054 ^b	0.003	0.001**

a, b means in the same row with different superscripts differ significantly (P < 0.01), SEM = Standard error of mean, units = (ppb) parts per billion, ** = (P < 0.01).

pig farmers used to source/purchase these feedstuffs. Samples were also collected from the farm feed stores. This was to determine the possible source of contamination of these feedstuffs, whether at source or farm store. The feedstuff samples were taken to the Department of Biochemistry Laboratory, University of Uyo, Uyo for aflatoxins level analysis. Before the laboratory testing, the feedstuff samples were milled, to a uniform 0.05 mm particle size [32].

2.4. Sample Preparation

An electronic scale G & G JJ 300 with capacity of 300 grams (d = 0.01g) was used to weigh out 5 grams of each of the feedstuff samples, after they were milled with an electric blender. After the milling of each of the samples, the blender was washed and allowed to dry before being used to mill another feedstuff sample. The milled feedstuff samples were put in plastic bottles, covered and kept in a cool dry place. The bottles are labeled accordingly.

2.5. Experimental Design

The experiment was carried out using a least square analysis in a randomized complete block design (RCBD).

2.6. Data Collection

Data were obtained from the aflatoxins laboratory analysis of the sampled pig feedstuffs, while data on the feedstuffs handling, storage and mould prevention methods adopted in the pig farms were obtained using a structured questionnaire. The questionnaire was validated by two experts in instrument measurement and evaluation from University of Uyo, Uyo. Each piggery farmer in the sampled pig farms in each of the six local government areas that constitute Uyo zone was given a questionnaire to fill and in cases where a question is not understood by the pig farmer, the researcher explained. The completed questionnaires were retrieved after the pig farmer has responded or collected at a later date by the researcher.

Table 4. Effect of feedtypes on Aflatoxins level of pig feedstuffs

Feedstuffs	Source	Aflatoxin level (ppb)	P-value
Cassava peels	Itu	0.032±0.003	0.157 ^{NS}
	Ibiono	0.042±0.002	
Cocoyam peels	Ibesikpo	0.031±0.001	0.522 ^{NS}
	Itam Mkt.	0.033±0.003	
Palm kernel cake	Akpan oil mills	0.034±0.002	0.283 ^{NS}
	Johnson oil mills	0.031±0.003	

SEM = Standard error of mean, units = (ppb) parts per billion, ** = (P < 0.01), CP = Cassava peels, BNW = Bambara nut waste, PKC = Palm kernel cake, BSG = Brewer spent grains.

Table 5. Effect of season on Aflatoxins levels of pig feedstuffs

Feedstuffs	Season		P-value
	Dry	Rainy	
Cassava peels	0.020±0.002 ^b	0.041±0.002 ^a	0.001**
Cocoyam peels	0.020±0.001 ^b	0.045±0.001 ^a	0.001**
Palm kernel cake	0.021±0.002 ^b	0.043±0.002 ^a	0.001**

SEM = Standard error of mean, units = (ppb) parts per billion, ** = (P < 0.01), CP = Cassava peels, BNW = Bambara nut waste, PKC = Palm kernel cake, BSG = Brewer spent grains.

3. RESULTS AND DISCUSSIONS

2.7. Statistical Analysis

The data obtained from this study for each individual feedstuff were analysed for effect of source, season, feedstuffs and farms location as well as the interaction effects of feedstuff and season, source and season and farm and season. Comparison among

The results of the effect of source of feedstuffs on the aflatoxins levels of pig feedstuffs is presented in Table 1. The results indicated that there were no significant (P > 0.05) differences in the aflatoxin levels of feedstuffs due to source of obtaining them. These results are in agreement with the findings [33,

Table 6. Effect of source and season on Aflatoxins levels of pig feedstuffs

Feedstuffs	Feed Source	Season		S.E.M	P-value
		Dry	Rainy		
Cassava peels	Itu	0.016	0.04	0.036	0.570 ^{NS}
	Ibesikpo	0.022	0.042	0.026	
Cocoyam peels	ItamMarket	0.018	0.044	0.003	0.910 ^{NS}
	Akpan Adem Market	0.02	0.048	0.002	
Palm kernel cake	Akpan oil mill	0.022	0.049	0.003	0.831 ^{NS}
	Wilson oil mill	0.02	0.042	0.002	

S.E.M = standard error of mean, units = (ppb): parts per billion, NS = not significant.

feedstuffs for aflatoxins level was carried out using analysis of variances (ANOVA) using Genstat computer package (Discovery edition 3). Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955). The results of the questionnaires responses of pig farmers were analyzed using descriptive statistics.

34, 18, 2], who reported the presence of aflatoxins in the range of between 28 - 46 ppb in all the feed and feedstuffs sampled in their respective studies from the sources of feedstuffs. The results indicated that there were no significant (P > 0.05) differences in the aflatoxins levels of the sampled pig feeding stuffs as a result of farm locations in the six local government areas of Uyo zone. The numerically high presence of aflatoxins in brewer spent grains may be attributed to

Table 7. Effect of feedstuff and season on the Aflatoxins levels of pig feedstuffs

Feedstuff	Season		S.E.M	P-value
	Dry	Rainy		
Cassava peels	0.021	0.04	0.003	0.728 ^{NS}
Cocoyam peels	0.02	0.048	0.003	0.727 ^{NS}
Palm kernel cake	0.024	0.04	0.003	0.728 ^{NS}

S.E.M = standard error of mean, units = (ppb): parts per billion, NS = not significant.

its high sugar and nutrients contents [35] which supports toxigenic moulds metabolism. It is also used as a culture medium for mushroom production, while cassava peels have the least aflatoxins level across the pig farm locations, probably due to the presence of cyanogenic glucosides which produces hydrogen cyanide (HCN) [36, 1], that may have interfered and discouraged the growth and development of toxigenic moulds. The results of this study is in agreement [2] but was not in agreement with the findings of [18, 37] that there were significant ($P < 0.05$) variations in the aflatoxins concentration levels with geographical locations, however this difference may be attributed to the similarity in the climatic/weather conditions of the different piggery farm locations in the areas sampled in this study.

In the dry season, aflatoxin levels were significantly lower than during the rainy season due to the weather conditions in the rainy season encourages toxigenic mould development [18, 23]. These are in support of the earlier findings [2, 29, 33, 37] who reported that aflatoxins concentration varies significantly ($P < 0.05$) with season, pointing out that wet season supports and encourages the growth and metabolism of toxigenic moulds which produces aflatoxins as metabolites.

The results indicated that there were highly significant ($P < 0.01$) differences on the main effect of feedstuffs on aflatoxins levels of pig feedstuffs. Cocoyam peels have the lowest value of 0.030 ppb, Cassava peels 0.040 was and palm kernel cake have the value of 0.042 ppb. This high level of aflatoxins in palm kernel cake may be attributed to the rich sugar and nutrients it contains which supports toxigenic moulds metabolism (aflatoxins contamination), it is also used as a culture medium for mushroom production, while cocoyam peels have the least aflatoxins level across the pig farm locations, this low level of aflatoxins may be attributed to the

presence of cyanogenic glucosides which produces hydrogen cyanide (HCN) [1, 36] which interfere and may discourage the growth and development of toxigenic mould.

The results indicated that there were highly significant ($P < 0.01$) differences on the effect of season on aflatoxins levels of the sampled feedstuffs except for brewer spent grain that is significant ($P < 0.05$). Cassava peels had the values of 0.020 ppb and 0.041 ppb in the dry and rainy season respectively. Cocoyam peels has the values of 0.020 ppb and 0.045 ppb, also palm kernel cake has the values of 0.021 ppb and 0.043 ppb respectively in dry and rainy seasons, respectively. The result was in agreement with the findings of [2] and other research findings as already stated above.

Cassava peels sourced from Itu have aflatoxins concentration values of 0.016 ppb and 0.040 ppb in the dry and rainy season respectively, while the cassava peels sourced from Ibesikpo have the values of 0.022 ppb and 0.042 ppb in the dry and rainy season respectively. Cocoyam peels sourced from Itam market have the values of 0.018 ppb and 0.044 ppb in the dry and rainy season respectively, while those sourced from Akpan Ndem market have the values of 0.020 ppb and 0.045 ppb in the dry and rainy season respectively. Also palm kernel cake sourced from Akpan oil mill have the values of 0.022 ppb and 0.049 ppb in the dry and rainy season respectively, while the palm kernel cake sourced from Wilson oil mill have the values of 0.020 ppb and 0.042 ppb in the dry and rainy season respectively. The result of the interaction of season and source of feedstuff indicated higher levels of aflatoxins concentrations at the sources of these feedstuffs in the rainy season than in the dry season, this further supports earlier findings that rainy season under which the identified conditions favourable for

aflatoxins production by toxigenic moulds is a common occurrence [36, 33, 20, 24].

The results indicated that there were no significant ($P > 0.05$) difference in the interaction effect of feedstuffs by season on the aflatoxins levels of pig feedstuffs. It follows that cassava peels have the aflatoxins concentration level of 0.021 ppb and 0.040 ppb in the dry and rainy seasons respectively, cocoyam peels have aflatoxins concentration level of 0.020 ppb and 0.048 ppb in the dry and rainy season respectively, also palm kernel cake have aflatoxins concentration value of 0.024 ppb and 0.040 ppb in the dry and rainy season respectively. The results of this study were in accordance with the findings of Jewers *et al.* (1986) and Ekwu (2014) [33, 2] who reported that the aflatoxins concentration varies with seasonal and geographical factors, it also supports the earlier work [33], who reported a seasonal peak in the occurrence of mycotoxins especially aflatoxins, adding that wet harvest seasons contributes to high levels of aflatoxins. The result further collaborated the findings [24, 20, 29] and they indicated that relative humidity of 80% and above, drought stress, temperature range of 27°C and above as well as the rate of precipitation which are all seasonal elements influences the aflatoxins concentrations level as well as mould development and growth in substrates.

4. CONCLUSION

This study was carried out to evaluate the impacts of season on aflatoxins loads of some pig's feedstuffs in the humid tropics in Niger Delta. The pigs feedstuffs are cassava peels, cocoyam peels and palm kernel cake are collected from two piggery farmers in each of the four LGAs that make up Uyo zone in both dry and rainy season. The study lasted for thirty-two weeks, the collected feedstuff samples were tested for aflatoxins concentration using AOAC method of thin-layer chromatography, the results from the study

indicated the presence of aflatoxins in all the feedstuff samples analyzed. It was evident that the feedstuffs used in feeding pigs in Uyo zone of Akwa Ibom State have aflatoxins levels that are within the tolerable limit which 20 ppb i.e. safe as at present, however if unchecked prolonged consumption of large quantities of these feedstuffs with low level of aflatoxins by the pigs may lead to aflatoxins related intoxications in pigs and pork consumers [18]. It also indicated that season have an effect on the aflatoxins level of pig's feedstuffs, with rainy season having higher levels of aflatoxins concentration than the dry season. It also shows that the piggery farm locations, source of feedstuffs, the interaction of season, source of feed and piggery farm locations have no significant ($P < 0.05$) difference on the aflatoxins levels.

From the results of this study, indicated the presence of aflatoxins in all the pig's feedstuffs sampled in this study. However this aflatoxins level is within the tolerable range ($< 20\text{ppb}$) at the moment, due to the fact that it cannot be totally eliminated, hence poses little or no health risks to pork consumers and pigs. But the key is to adopt measures that will keep it at this level or even below, in order to avoid the endangering of both animal and human health with high levels of aflatoxins. The results indicated that the level of aflatoxins varies with respect to the season, rainy season favour mould metabolism, growth and development and the corresponding aflatoxins production unlike in dry season. Hence adequate measures should be adopted in this season to check mate aflatoxins loads of feed ingredients and feeds.

It is therefore suggested that regular survey of aflatoxins loads and exposure of pigs and pork consumers be investigated at regular intervals such as bi-annually to monitor and safeguard livestock

production as well as public health. The followings are therefore recommended:

- i. Piggery farmers should be sensitized through workshops and seminars on the effects of feeding mould contaminated diets to pigs, while noting that humans i.e. pork consumers are at health risk and in the receiving end.
- ii. More awareness should be created and piggery farmers should be encouraged to use mycotoxin binder and deactivator/adsorbent in pig rations formulation, as a safety measure.
- iii. Improvement on the feeds storage methods such as using plastic airtight drums with cover to avoid absorption of moist, or by using rice husk and ash to cover the surface of the feedstuffs in order to reduce microbial or fungi contamination and growth.
- iv. Crop farmers should timely harvest and dry cereal grains before storage as improperly dried grains will support mould growth and development with 18 % moisture level.

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NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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