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Case Report
Resurgence of Aflatoxicosis and the Role of Physical Exercise in Aflatoxin Metabolism in Laying Hens

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Abstract

Background and Objective: Aflatoxins are among the top common courses of aflatoxicosis in poultry. In addition to the huge economic implications of aflatoxicosis to the poultry industry, aflatoxins are life-threatening to birds when present above the standard tolerable limit in poultry feed. Despite several reports on the levels of aflatoxin present in tested feed samples, natural cases of aflatoxicosis in poultry are rarely reported. The present study was conducted to report a natural case of aflatoxicosis in two different poultry farms where laying hens were fed with an aflatoxin contaminated feed. **Materials and Methods:** We collected a sample of poultry feed suspected to be contaminated with aflatoxins for analysis. Direct competitive ELISA using AgraQuant® total aflatoxin (TA) test kit was used to quantify the total aflatoxin level in the collected feed sample. **Results:** Laboratory analysis of the feed sample showed a high level of total aflatoxin of up to 320 ppb (parts per billion). This is 15 times above the FAO action point (20 ppb) for total aflatoxin in finished poultry feed. A post-mortem examination of the affected birds revealed typical lesions associated with aflatoxicosis. Interestingly, hens raised in a deep litter system showed more resistance to the same level of aflatoxin exposure when compared to hens raised in the cage system. **Conclusion:** We conclude that there is a surge in the natural cases of aflatoxicosis in poultry due to high level of aflatoxins in poultry feed, especially in developing tropical countries like Nigeria. We postulate that physical exercise could affect the rate of aflatoxin metabolism in poultry.

Key words: Aflatoxicosis, laying hen, mycotoxins, physical exercise, poultry feed

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins are the most commonly encountered sources of aflatoxicosis in poultry[1]. They are a group of mycotoxins which are toxic secondary metabolites produced during the degradation of feed components (maize, soya and groundnut) by fungal molds[2]. These molds mainly consist of *Aspergillus flavus*, *A. parasiticus*, *A. nominus*[3] and *Penicillium puberulum*[4]. Aflatoxins deteriorate the health and welfare of birds. The consumption of aflatoxins in high amount result in the death of exposed birds[5,6]. Aflatoxin contamination of feed is nowadays largely seen as a problem of tropical and sub-tropical regions of the world[6,7]. In the past, contamination of feed with aflatoxins especially maize has been a recurring event across the globe[3]. Despite several reports on the high level of aflatoxins detected in poultry feed samples and feed ingredients tested from different parts of the world,[2,8-12] only a few natural cases of aflatoxicosis in poultry resulting from the ingestion of aflatoxins in the poultry feed have been reported[13]. This could in part be due to the advancements in food storage and feed formulation techniques. Recently the availability of commercially formulated anti-toxin products known as "toxin binders" has also helped in reducing the occurrence of aflatoxicosis in poultry. Toxin binders are mostly made from herbs, spices and synthetic chemicals that are known to have mostly some liver protective effects[6,14]. However, the current feed crisis in Nigeria has increased the competition for maize and other protein sources particularly GNC (groundnut cake) among the different users of these feed components, which forms the greater percentage of the poultry feed. This competition has resulted in a sudden increase in the price of commercially formulated poultry feed and feed ingredients. Thus, forcing feed millers to use cheaper albeit substandard ingredients to reduce the cost of production. More also, the price of the commercially available toxin binders has increased just like the other feed ingredients, hence feed mills simply choose not to incorporate the toxin binders into their feed to maximize their profit margin. The difficulty and high cost of securing foreign exchange specifically the US dollar (USD = $) has contributed to the hike in the price of toxin binders and other imported feed ingredients such as the premix, usually imported from other countries around the world. At the level of the farms, poultry farmers producing their feed tend to simply ignore toxin binders, thus underestimating the adverse effects and economic implications of aflatoxicosis in poultry production.

The level of aflatoxins in livestock feed has been on the rise in most African countries apart from South Africa. Surveys conducted by the animal nutrition giant Biomin® revealed severe and extreme levels of aflatoxins in poultry feed between 2013 and 2016[15]. A follow-up survey by Biomin® also revealed a sharp rise in aflatoxins and other mycotoxins levels in maize and soya beans. This recent survey was conducted across 54 countries around the world between January to March 2017 and investigated both finished feed and raw materials used in formulating poultry feed[12]. However, the levels of aflatoxins detected in feed samples from Africa were rated moderate in 2017, unlike the extreme and severe ratings previously seen in the survey from 2013-2016. This indicates some level of progress in livestock feed quality with regards to aflatoxins contamination.

Many adverse effects have been linked to aflatoxicosis in poultry. These include a severe drop in egg production, low feed intake leading to weight loss and in chronic cases, liver and kidney damage, paralysis and death. Some forms of aflatoxins notably Aflatoxin M1 have been traced in animal meat and milk[16]. Of major concern in a country like Nigeria where there are no clear regulatory limits of aflatoxin levels for poultry feed is the tendency of a buildup of aflatoxin residues in poultry product. These residues expose consumers of poultry meat and products to a risk of aflatoxin toxicity. Despite the improvements in the methods for the detection of total aflatoxin (TA) in feed samples, there is a paucity of information on aflatoxicosis in laying birds, especially in the metabolism of aflatoxins in this group of poultry[17]. Most studies on aflatoxins are done in broiler chickens that are known to have a fast growth rate and high metabolic rate. The present study was conducted to report a natural case of aflatoxicosis in two different poultry farms where laying hens were fed with an aflatoxin contaminated feed supplied by a poultry feed miller. The relationship between the rates of aflatoxin build-up and aflatoxin toxicity among different groups of birds reared under different housing systems was also reviewed. We hypothesize that physical exercise plays a positive role in slowing down the rate of aflatoxin build-up by increasing the rate of aflatoxin detoxification in affected birds.

MATERIALS AND METHODS

Case history: Two poultry farms located in Sokoto, Northwestern Nigeria with a total of 5060 30-weeks old apparently healthy laying hens reported several mortalities to the Usmanu Danfodiyo University Veterinary Teaching Hospital, Sokoto. The pattern of mortalities of birds is shown in Fig. 1 and 2, for farm one and farm two respectively. In the first farm, 1500 birds were caged and had started laying 8 weeks before the incidence with a 65% egg production rate. Main observations in the affected birds include sudden

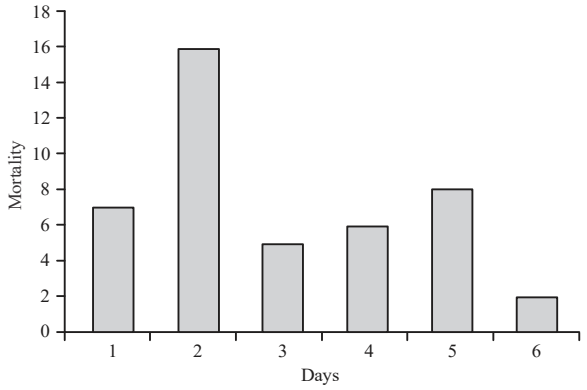

Fig. 1: Graphical representation of the mortalities recorded in the first 6 days in farm 1

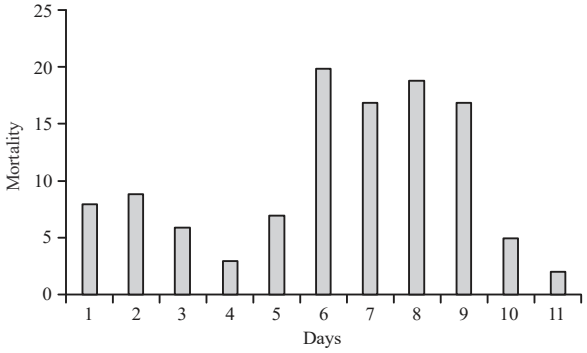

Fig. 2: Graphical representation of the mortalities recorded in the first 11 days in farm 2

mortalities, thin eggshells, cracked eggs, a severe and acute drop in egg production (from 65% down to 35%), reduction in egg size and paralysis (7 paralyzed birds). Reduced water intake was also observed (a drop from 420-400 L day^{-1} and later 360 L day^{-1}). A small number of birds (200) were kept in the rearing house on a deep litter system.

In the second farm, sudden mortalities of birds were recorded 48 hours after the introduction of the suspected feed. The mortalities increased daily until it reached a peak (Fig. 2). The farm had a flock size of 3360 apparently healthy 30-weeks old laying hens. Egg production at the time the contaminated feed was introduced was 54.3%. Six days later, the egg production dropped to 38%. Cracked eggs, reduced egg size and a decrease in the total daily egg production were observed.

Both farms 1 and 2 purchased the same feed from the same supplier with the same batch number. The farms are located 45 km away from each other. Some other farms that used the same feed with the same batch number also reported mortalities at the time when the birds were fed with the contaminated feed. All birds were fed with 110 g of feed per day and water was provided at libitum. All the birds were vaccinated against common poultry disease in their locality. No mortality or other sign of illness or abnormal behavior was recorded in the flocks prior to the consumption of the contaminated feed.

Table 1: Standards shows the OD of the total aflatoxin standards and the corresponding aflatoxin levels as well as the OD of the feed sample

x-axis	y-axis	Sample	OD
0	2.172	Poultry feed	0.305 (1/10th)
4	1.797		
10	1.056		
20	0.498		
40	0.238		

Sample collection: One hundred gram of feed sample was collected randomly from one of the sacks of the commercial feed. The collected sample was neatly sealed in a clean polythene bag and shipped to the laboratory for analysis.

Parameter measured

Aflatoxin quantification: Direct competitive ELISA technique using AgraQuant® total aflatoxin (TA) test kit (Romer Labs, Austria) was the method adopted for quantifying the total aflatoxin content of the feed. Briefly, 20 g of a representative sample was mixed with 100 mL 70% methanol for extraction. After filtration, 100 µL of the filtrate was further diluted in 900 µL of 70% methanol. 100 µL of diluted methanol extract was pipetted into the dilution well strip and carefully mixed with the 200 µL of conjugate present in the well strip. 100 µL of the mixture was immediately transferred into the antibody-coated microwell strip and incubated at room temperature for 15 min. The content of the microwell strip was discarded and the strip carefully washed 5 times. 100 µL of the substrate was added to the microwell strip and incubated at room temperature. After 5 min of incubation, 100 µL of stop solution was added and a microwell reader (Biotek ELX 800 Absorbance Microplate Reader, USA) was used to read the intensity with a 450 nm filter and 630 nm differential filter. The Optical Density (OD) obtained was compared with the reference standards (in a dose-response curve of the 5 standards) (Table 1).

RESULTS

Clinical investigation: An initial investigation by the local veterinarians on both farms revealed an enlarged friable

liver with damaged kidneys (Fig. 4). Aflatoxicosis was not considered initially because of the rare reportage of its occurrence in the region. When the commercial feed (which tested positive to aflatoxins) was changed after the sudden onset of mortalities, a sudden drop in mortality was noticed 24 h following the introduction of a new (uncontaminated) feed. The sudden high mortality initially seen and the sudden drop in mortality recorded after feed change caused a strong suspicion of the presence of a possibly toxic substance in the feed. Sample of the feed was collected and sent to the laboratory for total aflatoxin quantification.

Total aflatoxin quantification standards: Since the amount of total aflatoxin in each standard is known, the total aflatoxin content of the sample was interpolated from the standard curve and multiplied by the dilution factor (of 10). The estimated level of total aflatoxin content in the feed sample was therefore found to be 320 ppb (parts per billion) (Fig. 3). This level is far above the action point (20 ppb) for total aflatoxin in finished poultry feed.

Post-mortem findings: Pathology related to the reported case was as a result of the ingested aflatoxins. With different results and conclusions documented from field trials, scientific and laboratory research, it must be noted that the effects of mycotoxins are very complex and it is possible that clinical signs and lesions differing from the ones observed in this case may also occur. There was a significant reduction in weight gain throughout the intoxication period (Fig. 4b). Weight-bearing difficulties resulting from leg weakness, reduced bone strength, short shank and leg deformity were all observed (Fig. 4a). Aflatoxins are known to have a hepatotoxic effect in chickens and known to have a hepato carcinogenic effect in exposed animals. In acute-subacute aflatoxicosis as in this case, the liver appeared enlarged, friable, with the gall bladder enlarged and filled with bile (Fig. 4c).

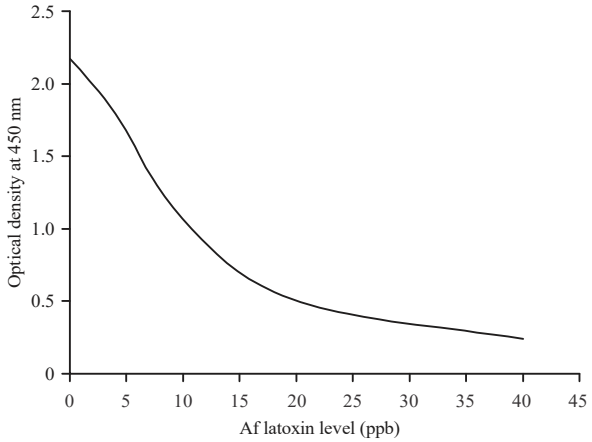

Fig. 3: Dose-response curve of the standards use for the total aflatoxin quantification

Fig. 4(a-c): Pre and post-mortem findings of some of the affected birds (a) Legs weakness as a result of reduced bone strength (b) Significant reduction in weight (c) Abdominal fat deposit, enlarged friable liver with gall bladder enlarged and filled with bile

Table 2: FDA guide on the maximum level of aflatoxin in poultry feed ingredients

Feed ingredients	Action level (ppb)
Groundnuts and groundnut cake (GNC)	20
Maize, groundnut products (GNC) and other feed ingredients (soya bean meal/cake) for immature poultry	20
Maize, groundnut and groundnut products (GNC) for Mature Poultry	100
Cottonseed meal*	300

*Indicates less commonly used ingredient in the Nigerian poultry feed industry, GNC is commonly used as a source of protein in poultry feed in Nigeria, Other ingredients like Soya bean meal/cake and fish meal are also used in feed formulation in Nigeria

DISCUSSION

Aflatoxins are immune-suppressive and are considered as the most toxic among all mycotoxins[6,11]. In addition to being immunosuppressive, they are also known to cause liver and kidney damage in birds[6]. It has been established that aflatoxins have several negative effects such as reduced feed intake, reduced weight gain and decreased feed consumption rate in poultry[17]. In addition it also leads to a sharp decline in egg production in laying birds[6]. All these result to severe negative economic consequences to the farmers[7,8,11]. Reduced feed intake by birds has been postulated to be a self-protective measure when they consume toxic substances. It occurs because of a negative feedback-like system in the major metabolic pathways involved in the detoxification process in the liver[17].

The level of total aflatoxin detected in the suspected feed sample was far above the maximum level allowed by the United States' regulatory body[18] in its action level guide on poisonous and deleterious substances in human food and animal feed[19] (Table 2). The level of the total aflatoxin in the tested feed sample was also far above the limit (20 ppb) set by the Nigerian national agency for food and drug administration and control (NAFDAC)[19], which is responsible for ensuring compliance by all commercial poultry feed millers in Nigeria.

An interesting observation in both affected farms was the absence of some apparent signs of aflatoxicosis in the uncaged group of birds. These birds were on a deep litter system and fed with the same feed that caused aflatoxicosis in the caged birds. The only sign observed in the uncaged group was a mild drop in egg production. Severe signs such as mortality and paralysis reported in the caged birds were not seen in the uncaged birds. This could be attributed to the level of exercise in the uncaged birds compared to the caged birds. This observation is similar to what has been reported in a planned experimental setting, where birds were grouped in different conditions to determine the effect of exercise on the toxicity of aflatoxins in poultry. Randall and Bird[17] observed that birds fed with aflatoxin containing feed up to 5 ppm (5000 ppb) plus repeated daily exercise using a treadmill had an overall weight more than birds fed aflatoxin containing feed (5 ppm) without daily exercise. Thus, indicating a better detoxication and tolerance in the group exposed to physical exercise.

Frequent exercise could help the uncaged birds in increasing the rate of aflatoxin metabolism and excretion. In addition, the cold temperature usually seen in the month of December during which the aflatoxin contaminated feed was fed to the birds could also increase the metabolic rates of the birds especially those in the deep litter system, leading to increased metabolism and detoxification of the aflatoxin. Wyatt *et al.*[20] in 1977 demonstrated that cold temperature enhances metabolism and low temperatures confer aflatoxin resistance to poultry. Exposure of animals to cold environment has also been shown to increase the metabolism of drugs[21] and by extension other toxic substances like aflatoxin. Also, birds on the deep litter system were shown to have low feed intake compared to birds in the cage system[22]. Low intake of the contaminated feed leads to low (more manageable) aflatoxin ingestion and a faster metabolism and excretion of the low level of ingested aflatoxin. Finally, individual variations among animals and humans also contribute to different level of tolerance of aflatoxin in the body seen among individuals and thus can affect their ability to detoxify ingested toxic compounds[17,23]. The indigenous free-range chickens are undoubtedly exposed to high levels of aflatoxins through the feed they scavenge on, which mostly consist of unwanted and low-quality grains not fit for human consumption[24].

CONCLUSION

In conclusion, physical exercise could play a lifesaving role in laying hens and other birds accidentally or experimentally exposed to aflatoxins even at a very high concentration of up to 5 ppm. This can be an additional benefit of the cage-free system of rearing poultry, which allows the birds to have as much physical exercise as possible unlike the cage system. Access to free movement and physical exercise could also be one of the reasons why aflatoxicosis is not commonly seen in birds reared under the traditional free-range system in most parts of Africa. The high level of total aflatoxin consumed by indigenous free-range chicken could also contribute to the general low weight gain observed in these birds, since aflatoxins are known to have a significant negative impact on the feed consumption and overall weight gain in poultry.

Currently despite having a national guide on the maximum tolerable limit of aflatoxin B1 in poultry and other animal feeds in Nigeria, the enforcement of this regulation is not adequate. It is recommended that, the local environmental factors in each country should be considered while setting up a national limit on the maximum tolerable limit of aflatoxin in poultry feed and when reviewing existing guidelines. A well formulated national or regional regulation would be better than the regulations adopted from other

countries or regions around the world. Proper monitoring and enforcements are also necessary when such regulations are in place to ensure compliance. Further experimental research to establish the exact mechanism of the role of physical exercise in aflatoxin resistance in poultry would shed more light on the aflatoxin resistance and exclude all possible confounding factors.

SIGNIFICANCE STATEMENT

This study reported natural cases of aflatoxicosis and identified the positive role of physical exercise in aflatoxin metabolism. This study will help the clinicians and farm managers in the identification and diagnosis of aflatoxicosis, which is often neglected in poultry disease management. Thus, this study sets the stage for further experimental research to establish the exact mechanism of the role of physical exercise in aflatoxin resistance in poultry.

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