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Assessing the prevalence of aflatoxin B1 in poultry feed in Dakar, Senegal: implications for animal and public health

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ABSTRACT

Aflatoxin B1 (AFB1) is a mycotoxin that can contaminate a wide variety of foodstuffs such as animal feed. Most of the ingredients used in livestock feed are highly susceptible to contamination, with a propensity to release residues in products intended for human consumption. In the case of the poultry industry, the ingestion of contaminated feed by poultry can lead to alterations in their sanitary and zootechnical performance. It also may cause a food safety problem related to the presence of mycotoxin residues in animal products. The objective of this study was to determine the prevalence and level of AFB1 in poultry feeds/ingredients used or marketed in the city of Dakar and its suburbs, and to discuss the potential risks to animal and human health. In total, 68 samples of starter, grower, finisher, pullet, and layer feeds and 2 ingredients samples were analysed. The research and quantification of AFB1 were done using the high-performance liquid chromatography (HPLC) method, as described in ISO 14718. The results showed that all (100%) of the samples were contaminated with levels ranging from less than 0.1 ppb to 52.3 ppb in the feeds. The level in ingredients samples were 0.5 and 177.5 ppb respectively for the fish flour and peanut meal. Of all the samples analysed (feeds and ingredients), 5.7% had contamination levels above the allowable limits for poultry feed. Given these results and the threat to public health posed by the increasing consumption of poultry products, it would be judicious to systematically include the research of mycotoxins in raw materials, along with the bromatological analyses used for food formation. Additionally, a study on mycotoxin residues in foodstuff from poultry would be of great interest for the protection of public health and food safety.

Keywords: Aflatoxin B1, Poultry feed, HPLC, Food safety, Public health, Senegal.

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INTRODUCTION

The importance of poultry farming in the global economy cannot be overstated, as it serves as a significant source of income for households around the world, especially in developing countries. In addition, poultry farming plays an essential role in meeting the daily protein needs of humans through the consumption of meat and eggs (Attia et al., 2022). For example, Dakar which is the Senegal capital city, houses most of this activity within a 100-kilometer radius (Ba et al., 2022) and a home to more than 80% of industrial poultry farmers (Traore, 2021). Despite the numerous advantages of this sector, there are several challenges that hinder its growth. One of the major obstacle is associated with poultry feed, the costs of which constitute a substantial portion of the total production costs in many West African countries (Chibanda et al., 2023a, 2023b). The diet of poultry primarily consists of cereals and its by-products, oilseeds and their by-products, fish meal, blood meal, minerals, and trace elements (FAO, 2013; Thirumalaisamy et al., 2016) . The raw materials used in poultry feed are either available locally or imported. Depending on the climate and storage conditions, these materials may be exposed to food contaminants such as mycotoxins (Guerre, 2016), resulting sometimes in a higher prevalence, especially in countries with hot and humid climatic conditions (Dieme et al., 2017).

Among the various mycotoxins, AFB1 is recognized as one of the most potent natural carcinogens, with the liver as its target organ (Wogan, 2000; Owaga et al., 2011; Wu et al., 2014). These mycotoxins have a detrimental effect on poultry health, the productivity of poultry farms, and their profitability (Yunus et al., 2011; Fouad et al., 2019). In addition to contaminated crops, AFB1 can also enter the food chain through animal-derived products such as eggs and poultry meat, posing a significant public health concern (Pandey and Chauhan, 2007; Wu et al., 2014). In Senegal, there is no specific legislation regulating the presence of mycotoxins in animal feed, and the standards defined by the European Commission are not always implemented. Furthermore, many individuals remain unaware of the potential hazards linked to consuming contaminated food and may lack information about mycotoxins (Ba et al., 2016). Investigating the prevalence of mycotoxins in animal feed appears very important and will enable animal producers to assess the risk associated with the use of certain food ingredients or feed from different regions (Murugesan et al., 2015).

To address this knowledge gap, the present study was undertaken with the primary objective of identifying and quantifying AFB1 in poultry feed samples collected from markets in Dakar and poultry farms in the peri-urban area. By providing comprehensive data on the quality of poultry feed, this study can also inform the development of policies and regulations for improved poultry feed, food safety and public health in Senegal.

MATERIALS AND METHODS

Ethics statement

Proceeding with the sampling process, prior consent was sought from the farmers. A comprehensive explanation of the study's objectives was provided to them, ensuring they had a clear understanding of the research goals and methods. This approach was undertaken to ensure transparency and to respect the participants' informed decision-making.

Study area

The present study was conducted in urban and peri-urban areas of Dakar (Figure 1) In urban area, samples were collected from the Tilène, Castor, Grand Yoff, and Fass markets. In the peri-urban area of Dakar, samples were collected from poultry farms. Subsequently, the samples were transported to the mycotoxin laboratory at the Dakar Food Technology Institute for analysis.

Sampling method

In the context of this study, due to logistical and financial constraints during the study, the non-probability convenience sampling method was used. The markets and farms were chosen for their accessibility and proximity. Thus, 100 samples of 500 g of feed were collected in accordance with the recommendations of ISO 14718 and stored in a cool and dry place for analysis. However, due to the limited availability of reagents, laboratory constraints, 70 samples were analyzed.

Laboratory methodology design

Detection and quantification of aflatoxin B1

The method used for extraction, purification, and quantification of AFB1 (Figure 2) in this study follows the ISO 14718 standard (ISO 14718:1998 Animal feeding stuffs - Determination of AFB1 content in compound feed - High-performance liquid chromatographic method). All requirements of the method were strictly fulfilled by the mycotoxin laboratory of the Food Technology Institute of Dakar. This technique is based on high-performance liquid chromatography and includes sample preparation steps such as grinding, extraction, and purification.

Sample grinding and extraction

In the laboratory, the samples were ground into a fine powder using a mini-mill. For extraction, 25.0 g of each sample's fine powder were placed in glass flasks and mixed with 12.5 g of celite. The sample-celite mixture was agitated before adding 12.5 ml of distilled water and 125 ml of chloroform. The mixture was manually agitated for one minute and then placed on a mechanical shaker for 30 minutes. Finally, the mixture was filtered using Whatman filter paper.

Purification of aflatoxin B1

Aflatoxin B1 purification using Florisil Column

The filtered mixture, comprising 25 ml was introduced into the Florisil column and C8 cartridge assembly. The assembly was rinsed with 5 ml of chloroform, followed by elution of AFB1 with 60 ml of acetone-water mixture. The eluate was collected in a round-bottom flask of a rotary evaporator heated to 40°C to 50°C until the acetone distillation ceased. One ml of methanol was added to the eluate in the flask and agitated with a vortex mixer to dissolve the aflatoxin residue on the flask walls. After adding 4 ml of water, the mixture was homogenized. The Florisil cartridge was disconnected and discarded, while the column was rinsed and kept for the subsequent purification step using the C18 cartridge.

Aflatoxin B1 purification using C18 Cartridge

The C18 cartridge was conditioned by passing 10 ml of methanol followed by 10 ml of water. The extract in the column and the flask were rinsed twice with 5 ml of methanol-water mixture (20/80). For elution, 2 ml of HPLC-grade methanol was used, and the eluate was evaporated under nitrogen gas. This method was used to purify and quantify AFB1 in the analyzed samples, following the ISO 14718 standard for determination of AFB1 in animal food using high-performance liquid chromatography.

Quantification of aflatoxin B1 by High Performance Liquid Chromatography (HPLC)

Each sample was extracted using a solvent solution (methanol/water). The sample extract was filtered and then transferred into an immunoaffinity column containing antibodies directed specifically against aflatoxins B1, B2, G1, and G2. The aflatoxins were eluted from the immunoaffinity chromatographic column using methanol. The quantification of aflatoxins was subsequently performed by high-performance liquid chromatography (reversed phase) with post-column derivatization by bromination followed by fluorometric detection. Post-column derivatization was carried out using potassium bromide.

The sample slated for analysis was propelled through a column filled with a finely

granulated stationary phase by a liquid known as mobile phase. The mobile phase is being a mixture of organic solvents and distilled water (water/methanol/acetonitrile, 13/7/4) containing 286 mg of potassium bromide and 152 µl of 4 M nitric acid. The flow rate was 1 ml/min for a column length of 25 cm, an internal diameter of 4.6 cm, and particle size of 5 µm. Aflatoxins are eluted within 16 min. The reference standard solution used contained AFB1.

Calculation and expression of results in aflatoxin B1 analysis of poultry feed in Dakar, Senegal

Calculation of aflatoxin B1 content in the aflatoxin B1 standard solution

The AFB1 content in the AFB1 standard solution was calculated using the following equation: $\rho = \frac{M*A}{d*k}$, where ρ is the AFB1 content in mg/ ml of the AFB1 standard solution, M is the molar mass in g/mol of AFB1, A is the absorbance, measured and corrected for the blank, d is the optical path length of the cuvette in cm, and k is the molar absorption coefficient of AFB1 in chloroform at 363 nm, in $mol^{-1} \cdot cm^{-1}$.

Calculation of the mass of aflatoxin B1 in the reference standard solution injected

The mass of AFB1 in the reference standard solution injected was calculated using the following equation $mc = f * \rho * Vic$ where m_c is the mass of AFB1 in the reference standard solution injected, f is the dilution and unit correction factor, ρ is the AFB1 content of the standard solution, and V_{ic} is the volume of the reference standard solution injected.

Calculation of the mass of aflatoxin B1 in the test solution

The mass of AFB1 in the test solution was expressed as $ma = \frac{As * 2mc}{Ac1+Ac2}$, where m_a is the mass of AFB1 in the test solution, As is the peak area corresponding to AFB1 in the test solution, m_c is the mass of AFB1 in the reference standard solution injected, A_{c1} is the peak area corresponding to AFB1 resulting from the previous injection of reference standard solution, and A_{c2} is the peak area corresponding to AFB1 resulting from the subsequent injection of reference standard solution.

Calculation of the aflatoxin B1 content in the sample

Finally, the AFB1 content in the sample was determined using the formula $Wa = \frac{ma * Vs * Vc}{ms * Vis * Vf}$, where W_a is the AFB1 content in the sample in µg/kg or ppb, m_a is the mass of AFB1 in the test solution, V_s is the volume of the undiluted sample extract used in the following procedure, m_s is the mass of the sample for testing, V_{is} is the volume of the sample extract injected, V_f is the volume of filtrate used for purification, and V_c is the volume of chloroform used for sample extraction.

Statistical analysis

The data obtained from the assays were processed in a Microsoft Excel version 2013 spreadsheet and subsequently transferred to RStudio software for statistical analysis. After determining the overall and specific prevalence, a generalized linear model was employed to analyze the AFB1 level in samples based on the type of food and the sampling location. When a significant difference was found, a pairwise comparison was done using the emmeans function under the emmeans package with the tukey method of p-value adjustment.

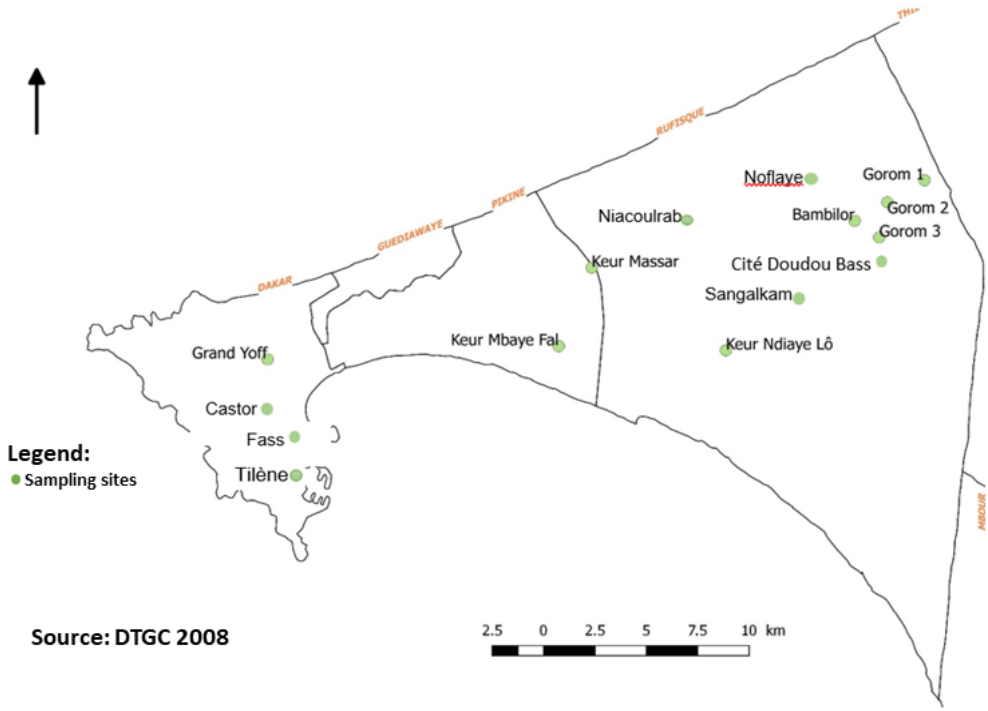

Figure 1: Map of the city of Dakar and locations where samples were collected.

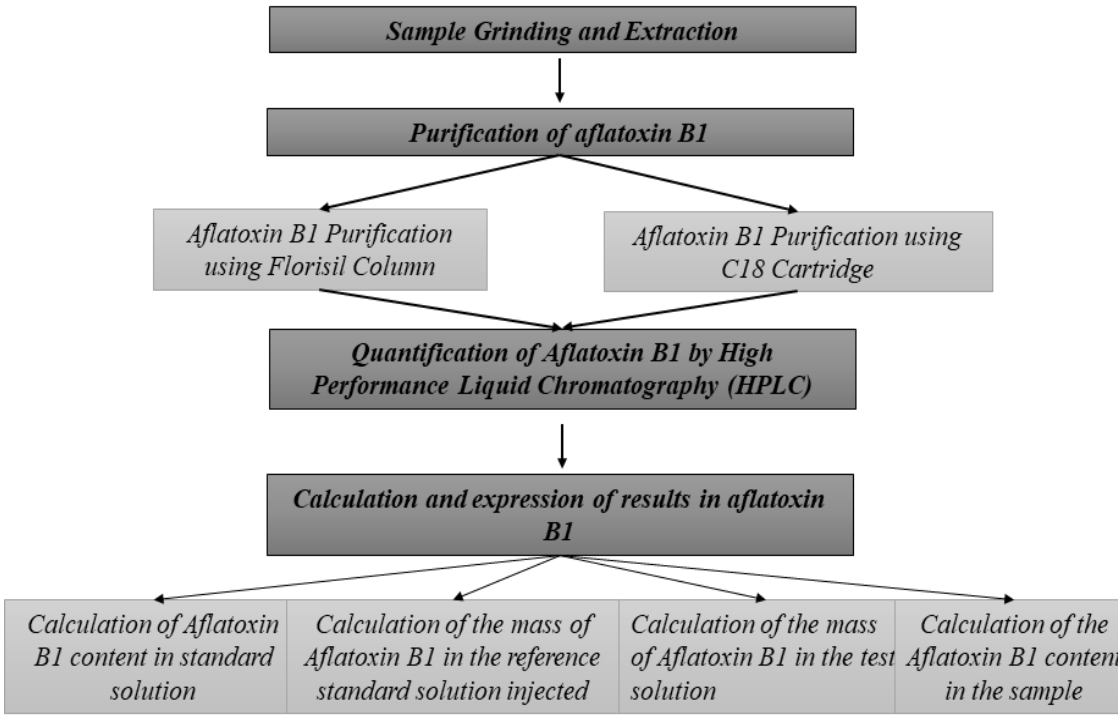

Figure 2: Laboratory analysis design flowchart.

RESULTS

Prevalence and levels of aflatoxin B1 Contamination in poultry feed samples

The results indicate a 100% overall contamination rate of poultry feed samples with AFB1. Out of all the analyzed feed samples, AFB1 was found in 40% (28/70) of the samples as traces (<0.1 µg/kg), while contamination greater than 0.1 µg/kg was detected in 60% of the samples. For samples with quantifiable levels of contamination, the mean concentration was 13.47 µg/kg, with a minimum of 3 µg/kg and maximum of 52.3 µg/kg. According to European Commission Regulation 2006/576/EC, the maximum allowable level of AFB1 in poultry feed is 20 ppb (µg/kg). Regarding this regulation, only 4.4% of the industrial feed samples exceeded the limit of 20 ppb (µg/kg) of AFB1, while 95.6% of the contained less than 20 ppb (Figure 3).

Factors of variation in aflatoxin B1 levels

The results of the generalized linear model showed that the level of the AFB1 did not vary significantly according to the sampling area whereas it varied significantly according to food type (χ^2= 208.340; df = 5; p < 2e-16 (Table 1). It was found to be significantly higher in peanut meal than the other feed type or ingredient (fish powder).

When comparing the contamination level by excluding the peanut meal and fish flour considered as ingredients, the analysis showed that there was no significant difference in the AFB1 level according to the feed type (χ^2= 3.9473; df = 4; p = 0.4131) (Figure 4; Table 2). According to sampling zone, the global statistics of the model showed a slightly significant difference (χ^2= 22.5020; df = 10; p = 0.0127) but when computing the pairwise comparison with the tukey method of P value adjustment, no significant difference were found.

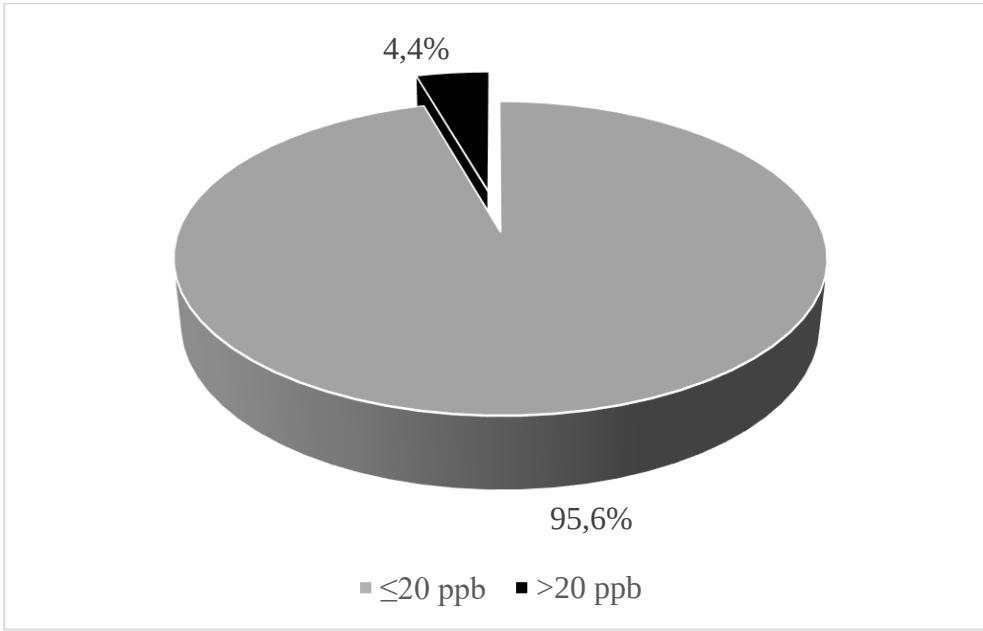

Figure 3: Repartition of samples according to the allowable level of AFB1 in poultry feed according to European Commission Regulation 2006/576/EC.

Table 1: Pairwise comparison of the aflatoxin B1 level according to the feed or ingredients type.

contrast	estimate	Standard error	df	t.ratio	p.value
Finisher - Fish flour	16.163	11.02	35	1.467	0.7615
Finisher - Grower	12.313	5.51	35	2.236	0.3034
Finisher - Layer	4.036	5.18	35	0.78	0.9855
Finisher - Peanut meal	-160.837	11.02	35	-14.6	<.0001
Finisher - Pullet	11.33	7.21	35	1.571	0.7008
Finisher - Starter	8.472	5.1	35	1.661	0.645
Fish flour - Grower	-3.85	10.82	35	-0.356	0.9998
Fish flour - Layer	-12.127	10.65	35	-1.138	0.9116
Fish flour - Peanut meal	-177.000	14.42	35	-12.272	<.0001
Fish flour - Pullet	-4.833	11.78	35	-0.41	0.9996
Fish flour - Starter	-7.692	10.62	35	-0.725	0.9901
Grower - Layer	-8.277	4.74	35	-1.747	0.5909
Grower - Peanut meal	-173.15	10.82	35	-16.007	<.0001
Grower - Pullet	-0.983	6.9	35	-0.142	1
Grower - Starter	-3.842	4.66	35	-0.825	0.9806
Layer - Peanut meal	-164.873	10.65	35	-15.478	<.0001
Layer - Pullet	7.294	6.64	35	1.098	0.9245
Layer - Starter	4.436	4.26	35	1.042	0.9403
Peanut meal - Pullet	172.167	11.78	35	14.619	<.0001
Peanut meal - Starter	169.308	10.62	35	15.95	<.0001
Pullet - Starter	-2.858	6.58	35	-0.434	0.9994

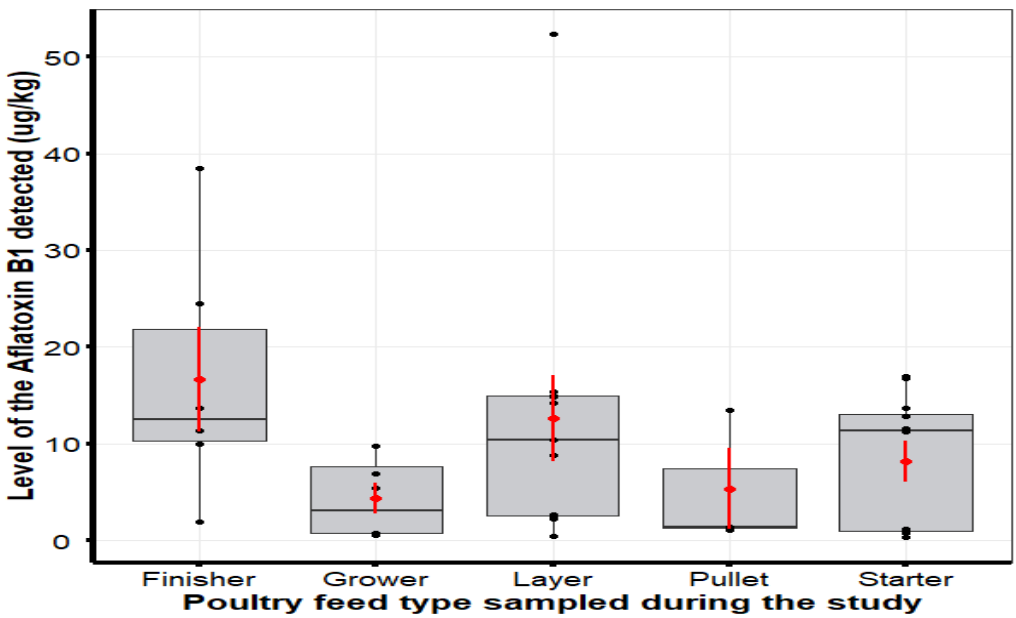

Figure 4: Comparison of the AFB1 levels between the different industrial poultry feed.
The boxplot shows the median, and upper and lower quartiles while the means and the standard errors are shown in red.

Table 2: Pairwise comparison of the AFB1 level according to feed type excluding the ingredients.

Contrast	Estimate	SE	df	t.ratio	p.value
Finisher - Grower	12.313	5.51	35	2.236	0.1907
Finisher - Layer	4.036	5.18	35	0.78	0.9348
Finisher - Pullet	11.33	7.21	35	1.571	0.5251
Finisher - Starter	8.472	5.1	35	1.661	0.4701
Grower - Layer	-8.277	4.74	35	-1.747	0.4199
Grower - Pullet	-0.983	6.9	35	-0.142	0.9999
Grower - Starter	-3.842	4.66	35	-0.825	0.921
Layer - Pullet	7.294	6.64	35	1.098	0.8063
Layer - Starter	4.436	4.26	35	1.042	0.8341
Pullet - Starter	-2.858	6.58	35	-0.434	0.9923

DISCUSSION

This investigation revealed a high prevalence of contamination as AFB1 was detected in all the collected samples. High level contaminations were previously reported (Gueye et al., 2022). Almost all poultry feed is contaminated with mycotoxins, probably due to the use of contaminated raw materials or inadequate storage of the feed (Devegowda and Murthy, 2005). Cereals and peanuts are particularly susceptible to contamination by toxin-producing molds in many countries and are commonly used as basic raw materials (Ahmadou, 2019; Magnin and Travel, 2016). In Senegal, the peanut and its derived products are very used in the food of the population or poultry and their cultivation is widespread (Gray, 2002) and generates 60% of the rural cash income and accounts for about 70% of the rural labor force (Ntare et al., 2005). Thus, peanut/groundnut are the most highly and frequently contaminated crops used for poultry feed (Benkerroum, 2020), which could explain the levels of aflatoxin contamination in the analyzed samples, especially since contamination can occur before harvest, in the soil, during drying or storage (Nakavuma et al., 2020). AFB1, the most toxic of the aflatoxins, is produced by molds belonging to the genus Aspergillus, which can more easily develop and produce toxins in hot and humid regions (Magnin and Travel, 2016). Furthermore, the prevalence of AFB1 may depend on the sensitivity/specificity of the method of determination. The HPLC used in this investigation can detect very low levels of mycotoxins. In accordance with regulations, 94.29% of the samples contain levels below the recommended maximum levels (in complete poultry feed - Regulation No. 2011/574/EC) of less than 20 µg/kg (ppb) of AFB1 (from less than 0.1 to 17 ppb). The treatment of ingredients during the feed manufacturing process influences the levels of mycotoxins they contain, in terms of reduction (Čolović et al., 2019). In Dakar and its peri-urban areas, most of the feed distributed to poultry are of industrial origin, and such feed is subjected to stricter treatment of the raw materials used. This treatment includes several techniques such as heat inactivation, irradiation, or treatment with oxidizing agents, acids, and/or bases (Leibetseder, 2006; Karlovsky et al., 2016; Sipos et al., 2021). However, 4.4% of industrial feed samples exceeded acceptable limits and the occurrence of chronic or acute toxicity in birds depend on the duration of exposure and body metabolism (Abedi and Talebi, 2015). The high levels of AFB1, exceeding 20 µg/kg, detected in this study suggest that the high contamination could be attributed to inadequate harvesting, poor processing techniques of raw materials, or poor storage conditions of both raw materials and foods after manufacture (Kumar et al., 2021). The presence of AFB1 in various types of feed ingredients and formulations poses a serious threat to poultry health with an increasing

susceptibility of birds to diseases and mortality (Fouad et al., 2019). Numerous studies carried out on various animal species have revealed the hepatocarcinogenic effects of aflatoxins through oral exposure, primarily from AFB1 (Fawaz et al., 2022). The chronic toxicity resulting from AFB1 ingestion is characterized by a loss of weight, and increase of the susceptibility to infections, a decrease in the average daily gain, as well as a drop in egg-laying capacity (Fouad et al., 2019). Despite the preventive measures implemented during the production process, crop contamination cannot always be avoided, as most mycotoxins are chemically stable and resistant to changes in temperature and storage conditions (Quillien, 2002). High doses of AFB1 can cause both acute and chronic intoxication in poultry, whether they are used for meat or egg-laying, even chickens are more resistant to acute aflatoxicosis than other poultry species, except during embryonic development (Monson et al., 2015). According to these authors, acute intoxication occurs when birds ingest a large dose of aflatoxin at one or more times, resulting in death within a time frame that varies according to their specific susceptibility. Beyond the health problems caused in poultry, AFB1 is also responsible for the decrease in zootechnical performance and significant economic losses (Pal et al., 2021). The consumption of contaminated feed for several weeks may result in chronic toxicity in poultry, with a significant negative impact on production and the economy, particularly in laying hens (Ditta et al., 2018; Fouad et al., 2019) as well as public health due to their residues in poultry products.

Moreover, mycotoxins have a worldwide public health problem, especially in developing countries where food safety or herbal medications regulations may be less strict (Wu et al., 2014; Ikeagwulonu et al., 2020). Indeed, mycotoxins can enter the human food chain directly through grains, seeds, spices, fruits, beverages, and other plant materials, and indirectly through food products obtained from animals fed with contaminated feed (Galvano et al., 2005; Aristil, 2019). The consumption of contaminated food with mycotoxins has been associated with an increase in liver cancer cases in populations in Africa and Asia (Liu and Wu, 2010; Meijer et al., 2021). In addition, infants and young children are particularly vulnerable to the effects of mycotoxins. Ingestion of mycotoxin contaminated food by children can retard growth and developmental delays, cognitive and neurological problems, as well as academic failures (Kadan and Aral, 2021). To mitigate the potential health hazards of mycotoxin-contaminated poultry and its products, several measures such hygienic harvesting, safe storage of raw material (ingredients), feed mill hygiene and the use of mycotoxin binders have been employed (Whitlow, 2006; Chulze, 2010; Kolosova and Stroka, 2012). However, the most effective way to minimize mycotoxin contamination in poultry products and protect public health is to implement good farming practices. This includes certifying the quality of raw materials, such as feed and bedding, and adhering to proper practices for the transformation and feeding of poultry. By implementing these measures, the poultry industry can reduce the risk of chronic toxicity in poultry, improve zootechnical performance, and prevent economic losses.

Conclusion

The objective of this study was to determine the prevalence of AFB1 in poultry farms, the levels of contamination. The findings of this study highlight a contamination of 100% of feed ingredients and suggest the need to strengthen the quality control of ingredients used in the formulation of poultry rations and manufactured feeds to minimize the risk of mycotoxin contamination in poultry products. The high contamination levels of peanut ingredients often utilized in poultry feed formulations increase the risk of aflatoxin transfer to human food, thereby highlighting the significance and relevance of this study. Furthermore, this study opens new possibilities for the investigation of mycotoxin residues in chicken carcasses and their by-products to ensure public health protection. Overall, these results underscore the importance of implementing comprehensive measures to reduce the risk of mycotoxin contamination in the poultry industry and safeguard public health.

AUTHORS' CONTRIBUTIONS

DLD and MN: conceptualization and design of the study; MN: collection of the samples and analysis in the laboratory; SM, DLD and KBA: supervision of the study; KBA, MN and DKM: Formal analysis, interpretation, and visualization of the data; KBA: Writing the first draft of the manuscript; DKM, SM, MN, and DLD: reviewed the first draft of the manuscript; All authors reviewed and approved the final draft.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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