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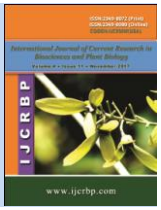

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Fungal Profile and Mycotoxin Contamination in Animal Feed in Urban and Peri-urban Zones of Bamako

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Abstract

In animal production in Mali, food appears to be the major constraint. In fact, mycotoxin contamination of animal feed is common and widely spread in West Africa. Due to their ubiquity, mycotoxin producing moulds are capable of reducing the nutritional value of animal feed by elaborating several mycotoxins. Animal feed contaminated with mycotoxins has adverse effects on animal health and productivity. Also, mycotoxins may be carried over into meat and milk when animals are fed with contaminated feed. Samples of feed used for animal nutrition in Urban and Peri-urban Zones of Bamako were randomly collected and analyzed for fungal flora and natural incidence of mycotoxins. Ten mould genera were recovered, six of them known to be mycotoxigenic. More than 11 species were determined. Fumonisins, deoxynivalenol and zearalenone were detected in all the samples, while Aflatoxins were not detected in samples from Massala. Thirty-six out of 36 samples were contaminated with zearalenone, 34 out of 36 were contaminated with Fumonisins and 26 out of 36 were contaminated with deoxynivalenol. Also, 7 out of 36 samples were contaminated with aflatoxins. This study indicates the need for continuous assessment of the mycological status of animal feed production, in order to ensure food safety.

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Introduction

The Malian herd is estimated at 9,721,328 cattle, 13,081,448 sheep and 18,216,005 goats (DNPIA, 2012). Food appears to be the major constraint to the development of animal production in Mali (IER, 2002). The quality of livestock feed and its availability to resellers cause enormous problems. If animal products constitute a lipidic, proteinic and carbohydrate contribution which is appreciable in food, their consumption and that of its derived products could have pathological consequences no less negligible for humans and livestock. Bacteria and fungi (moulds) naturally occurring toxicants contaminate foods and feeds. These food and feed are behind serious health risk to animals. Some fungi produce mycotoxins, which ingestion can cause diseases called mycotoxicoses. Mycotoxins can be produced in either pre-harvest or post-harvest stage of the crop.

In addition to their negative impact on agricultural production (Kana et al., 2013; Rodrigues et al., 2011), nutritional and organoleptic properties; moulds can also synthesize different mycotoxins (Shareef, 2010). It is well known that contamination of animal feed with mycotoxins may induce sanitary disturbances and mortality among animals and secondary contamination of human consumers via eggs, meat, or milk (Nyamongo and Okioma, 2005).

Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic toxic effects (Rodrigues and Naehrer, 2012; Binder et al., 2007). Mycotoxins exhibit toxic actions and are characterized by carcinogenic, mutagenic, teratogenic, and estrogenic properties (Cegielska-Radziejewska et al., 2013). Most toxic species belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* (Pitt and Hocking, 2009). Mycotoxins such as aflatoxins, zearalenone, deoxynivalenol, fumonisins, and patulin can be considered the most common ones found in feed and food (Keita et al., 2013; Koné et al., 2016).

Most mycotoxicoses of animals are caused by an intake of low concentration of contaminants over a long period lead to the typical chronic symptoms of poor growth, poor feed efficiency, and suboptimal production. 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 (Cegielska-Radziejewska et al., 2013; Mabbett, 2004).

In Mali, less or no information exist on the fungal status including mycobiota and mycotoxins in food and animal feed (Keïta, 2013). For quality control the identification of the contaminating mycobiota is essential because it provides data on the potential production of its mycotoxins and is a helpful indicator to determine feed hygienic quality (Saleemi et al., 2010).

The aim of the present study was to determine the occurrence of mycotoxicogenic fungi and mycotoxins in animal feed in urban and peri-urban zones of Bamako, not previously studied. Samples were collected from the central region, particularly from Bamako (the largest producing area of Mali). The study includes enumeration and identification of mould genera and species, natural levels of mycotoxins such as aflatoxin, fumonisin, deoxinivalenol (DON), and zearalenone (ZEA).

Materials and methods

Samples

A total of 36 representative samples (1-2 kg per sample) of six commercially prepared animal feeds were collected from Sala, Tienfala, Tlomadio and Massala in Koulikoro region (Mali), during 2010. All samples were homogenized and divided to obtain a 1 kg of working sample for analysis. Each sample was ground in a laboratory mill. The main component of samples was corn followed by deactivated soybean, soybean pellet, wheat bran, sorghum, animal proteins, and grain mill products. For mycological examination feed samples were immediately analyzed upon arrival or they were stored for 2-3 days in paper bags at room temperature (about 25°C). Feed samples intended for mycotoxin analysis were stored at −20°C.

Enumeration and isolation of fungi

For the enumeration and isolation of fungi in the animal feed samples, the dilute plate technique was used Pitt and Hocking (2009). For that, ten grams of each homogenized feed sample were mixed with 90mL of 0.1% peptone and shaken on a horizontal shaker for 20 minutes. After this treatment, 0.1mL of this dilution was inoculated on each of three different media: Dichloran Rose Bengal Chloramphenicol Agar (DRBC) to enumerate total culturable fungi; Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) Czapeck-Dox Agar (CDA), Sabouraud Dextrose Agar (SDA) to isolate and identify the different fungi. For selective isolation of *Fusarium* species, Dichloran Chloramphenicol Peptone Agar (DCPA) was used and plates were incubated under 12 hours of light: 12 hours of darkness photoperiod, at 25°C for 7 days. Plates containing 10–00 colonies were counted and results were expressed as colony-forming units per gram of sample (CFU/g) [10]. Representative colonies of each type were purified by subculturing them onto plates with PDA. Filamentous fungi were identified at genus level according to macro- and microscopic criteria in accordance with Samson et al. (2004). Fungal isolates were identified at species level according to Pitt and Hocking (2009), Nelson et al. (2009) and Simmons (2009).

According to Gonzalez et al. (1995), Pacin et al. (2003), and Saleemi et al. (2010) cited by Greco et al., 2014); the isolation frequency (Fr) and relative density (RD) of genus/species were calculated as follows:

$$Fr\ (\%) = \frac{\text{Number of samples with a genus or species}}{\text{Nunber of samples}} \times 100$$

$$RD(\%) = \frac{\text{Number of isolates of a genus or species}}{\text{Total number of fungi isolated}} \times 100$$

Aspergillus and *Penicillium* isolates were preserved on agar slants of MEA. While *Fusarium* isolates were preserved on Potato Dextrose Agar (PDA) at 4∘C and in 20 %glycerol at −20°C.

Mycotoxin analysis

To evaluate mycotoxin occurrence, feed samples were subjected to quantitative analysis using ELISA-based analytical test kits for aflatoxin, ochratoxin A, fumonisin, deoxynivalenol, and zearalenone (RIDASCREEN FAST, R-Biopharm AG). The extraction procedures were according to manufacturer's protocol. In brief, 5 g of ground sample was extracted with 25mL of 70% methanol for aflatoxins, ZEA, and fumonisins. For OTA and DON, samples were extracted with 12.5mLof 70% methanol or 100mL of distilled water, respectively. Afterwards, samples were shaken vigorously for 3 minutes and the extracts filtered through Whatman No1 paper. Then, aflatoxins, OTA and ZEA filtrates were diluted with distilled water in the ratio 1 : 1 and fumonisin filtrates in the ratio 1 : 14. Fifty μL of the diluted filtrate per well was used for testing (Greco et al. 2012).

Statistical analysis

The Analysis of Variance test was used to analyze fungal counts and toxins content. Multiple Range Test for variables was employed to compare means of fungal counts and toxins content of samples from different villages. The SAS software was used for all analyses and differences were considered significant when $p<0.05$.

Results

Mycotoxin production in animal feed, as a consequence of fungal contamination, is one of the major threats to human and animal health (Castillo et al., 2004). In our study, all the samples; from the villages of Sala, Tienfala and Massala; analyzed were contaminated with fungi and mycotoxins. In average, fungal counts of 2.59 10^5 CFU/g, 1.59 10^5 CFU/g and 2 10^4 CFU/g were obtained respectively from Sala, Tienfala and Massala animal feed samples (Fig. 1A).

The analysis of results in Fig. 1, shows that animal feed samples from Sala were the most contaminated with fungi (98% more than total fungi in animal feed samples from Tienfala and Massala). According to Gimeno (2002), the mycological quality of these animal feed can be qualified as good for animal feed sample from Massala (count range <3.10^4 UFC/g), and bad for animal feed samples from Sala and Tienfala (count range >7.10^4 UFC/g). On both media used for microbial count, fungal count range was 0–10^5 CFU/g.

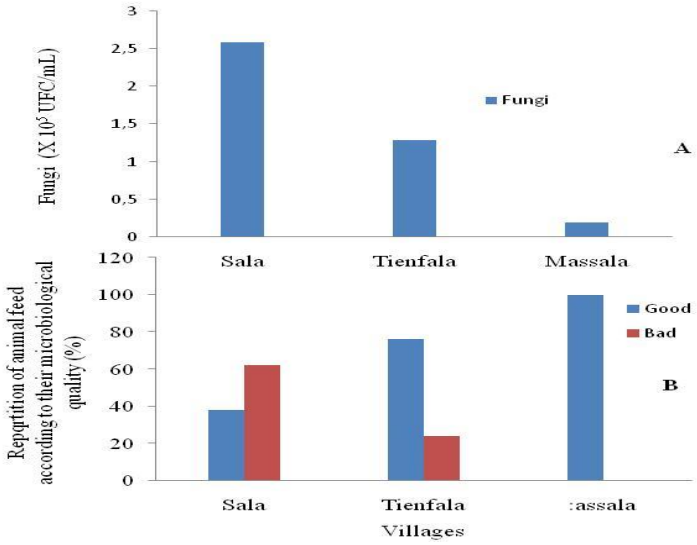

Fig. 1: Contamination of animal feed samples with fungi (A) and repartition of animal feed according to their microbiological quality (B).

According to the mycological quality criterion 38% of animal feed samples from Sala, 75% from Tienfala and 100% from Massala could be qualified as good and 62% of animal feed samples from Sala and 25% from Tienfala could be qualified as bad (Fig. 1B).

In this study ten mould genera were recovered, four of them known to be mycotoxigenic (Pitt and Hocking, 2009). The frequency (Fr) and relative density (RD) are showed in Table 1. Analysis of results in Table 1, showed that *Aspergillus* (100%) *Cladosporium* (100%), *Fusarium* (100%), *Rhizopus* (100%) and *Mucor* (100%) were the most predominant genera determined in the animal feed samples studied.

Table 1. Fungal genus present in animal feed samples.

Genus	Number of isolates	Fr (%)*	RD (%)**
Aspergillus	36	100	16.8
Bipolaris	13	36.1	6.07
Botritis	3	8.3	1.4
Cladosporium	36	100	16.8
Fusarium	36	100	16.8
Mucor	36	100	16.8
Rhizoctonia	14	38.9	6.5
Rhizopus	36	100	16.8
Trichoderma	2	5.6	0.9
Trichophyton	2	5.6	0.9

*Isolation frequency; **Isolation relative density.

While the predominant genera are coincident with the above mentioned results, the most frequent mycotoxigenic fungi were those from the genus *Aspergillus* and *Fusarium* (100%), followed by *Rhizoctonia* (38.9%), *Bypolaris* (36.1%), *Botritis* (8.3%), *Trichoderma* (5.6%) and *Trichophyton* (5.6%), although the presence of *Eurotium* spp. was not previously reported. Seventeen (17) species were recovered from the animal feed samples studied. Several species from different genera were determined in each sample. The frequency (Fr) and relative density (RD) are showed in Table 2.

Table 2. Fungal species present in animal feed samples.

Species	Number of isolates	Fr (%)*	RD (%)**
Aspergillus fumigatus	36	100	10,3
Aspergillus flavus	15	41,7	4,3
Aspergillus japonicus	21	58,3	6
Aspergillus parasiticus	10	27,8	2,9
Aspergilus niger	36	100	10,3
Bipolarias oryzae	13	36,1	3,7
Botritissp	3	8,3	0,9
Cladosporium	36	100	10,3
Fusariumgraminearum	36	100	10,3
Fusarium oxysporium	16	44,4	4,6
Fusarium spp.	36	100	10,3
Mucor sp.	36	100	10,3
Rhizoctonia sp.	14	38,9	4
Rhizopus spp.	36	100	10,3
Trichoderma harzianum	2	5,6	0,6
Trichoderma spp.	2	5,6	0,6
Trichophyton equinum	2	5,6	0,6

*Isolation frequency; **Isolation relative density.

The most prevalent mycotoxigenic *Fusarium* species: *Fusarium graminearum* (Fr 100%-RD 10.3%) and *Fusarium spp.* (Fr 100%-RD 10.3%), and *Aspergillus* species: *Aspergillus fumigatus* (Fr 100%-RD 10.3%) and *Aspergillus niger* (Fr 100%- RD 10.3%) were determined. On the other hand, we found *Aspergillus*,

Cladosporium, *Fusarium*, *Rhizopus* and *Mucor* species, followed by *Rhizoctonia*, as prevalent mycobiota (Tables 1 and 2). *Bipolaria* (Fr 36.1%), *Botritis* (Fr 8.3%), *Trichophyton* (Fr 5.6%) and *Trichoderma* (Fr 5.6%) were also found.

Table 3 shows the distribution concentration of mycotoxins in the poultry feed tested in this study. ZEA were detected in all the samples analyzed in a range of 75–115 ppb. Thirty four out of thirty six (36) samples (94%) were contaminated with fuminosin and 24 out of 36 animals feed samples studied (67%) were contaminated by OTA. But only 8 out of 36 samples (22%) were contaminated with aflatoxin B1 (Table 3). Thus, aflatoxins occurred less frequently in animal feed in studied samples compared to other mycotoxins. Aflatoxin values ranged between 3.4 and 47.9 ppb. Fumonisin B1 levels ranged between 4000 and 5.740 ppb. Zearalenone levels ranged between 75 and 115 ppb.

Table 3. Concentration of different mycotoxins in animal feed samples**.**

Villages	Parameters	Mycotoxins			
		Aflatoxin	DON	Fumonisin	ZEA
Sala	Number of samples	3/10	7/10	10/10	10/10
	Positive tested	(30%)	(70%)	(100%)	(100%)
Tienfala	Number of samples	4/16	12/16	14/16	16/16
	Positive tested	(25%)	(75%)	(88%)	(100%)
Massala	Number of samples	0/10	7/10	10/10	10/10
	Positive tested	(0%)	(70%)	(100%)	(100%)

Discussion

All the samples; from the villages of Sala, Tienfala and Massala; analyzed were contaminated with fungi. We observed significant differences ($p< 0.05$) among fungal contamination levels in feed from villages, while non-significant differences were found between feed samples from each village. These results indicate the need for continuous assessment of the mycological status of animal feed production. Fungal counts were in the range 2×10^4 to 2.59×10^5CFU/g. Xerophilic fungi, which are capable of rapid growth above about 0.77 water activitycan cause feedstuff spoilage(Pitt et Hocking, 2009). So much care must be made on storage conditions while keeping animal feed, as the humidity increases fungal growth and proliferation of xerophilic fungi can occur.

In this study, cotton and rice bran were the main component of animal feed in the samples analyzed. In this study ten mould genera were recovered, four of them known to be mycotoxigenic. *Aspergillus* and *Fusarium* were the most predominant genera determined followed by *Rhizoctonia* (38.9%), *Bypolaris* (36.1%), *Botritis* (8.3%), *Trichoderma* (5.6%) and *Trichophyton* (5.6%). This result is in accordance with those of many researchers who showed the occurrence of *Aspergillus* spp. and *Fusarium* spp. are widespread, mainly in tropical countries (Kana et al., 2013; Greco et al., 2014; Kehinde et al., 2014). In accordance with previous studies in Africa *A. fumigatus, Aspergillus niger, A. parasiticus* and *A. flavus* were the aflatoxigenic species isolated. *Fusarium graminearum* and *Fusarium oxysporium* were the most *Fusarium* species recovered (Filtenborg et al., 1996; Bhattacharya and Raha, 2002; Kehinde et al., 2014). Magnoli et al. (2002), have shown that most poultry feeds are contaminated with species belonging to *Penicillium*, *Fusarium* and *Aspergillus* genera. According to the mycological quality criterion (Gimeno, 2002), 38% of animal feed samples from Sala, 75% from Tienfala and 100% from Massala could be qualified as good and 62% of animal feed samples from Sala and 25% from Tienfala could be qualified as bad.

In this study, all the analyzed animal feed samples were contaminated with at least two different mycotoxins. Several studies report the simultaneous occurrence of two carcinogenic mycotoxins. Aflatoxin B1 and fumonisin B1, was determined in feed intended for animal consumption. In this work co-occurrence of mycotoxins was determined in all of the samples analyzed. Co-occurrence of at least three out of four mycotoxins was determined in all of the samples analyzed. Co-occurrence of four mycotoxins was determined in 72% (26/36) of the samples. Greco et al. (2014), while studying mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals detect co-occurrence of mycotoxins in all the samples analyzed. Aflatoxins occurred less frequently in animal feed in studied samples compared to other mycotoxins. But, the combined action of mycotoxins can generate an

interactive effect such as additivity, synergism or antagonism. Synergistic effects in fattening poultry were observed in the case of aflatoxins and OTA. This synergistic interaction causes the most toxic effects (Greco et al., 2014). An additive effect of aflatoxins and DON in fattening poultry, which causes reduction of body weight and increased mortality, has been demonstrated. In our study co-occurrence of aflatoxins and DON was observed in 26 out of 36 samples (72%). Simultaneous occurrence of two carcinogenic mycotoxins, aflatoxins and fumonisins was recorded in 31/36 (86%) of the samples.

Conclusion

In this work, all the animal feed samples analyzed were contaminated with mycotoxins. The co-occurrence of several mycotoxins was also demonstrated. Also, in order to guarantee the hygienic and nutritional quality of feed to ensure animal health and prevent human food-borne diseases, we suggest periodic monitoring and the application of the Hazard Analysis and Critical Control Points (HACCP) on the prevention and control of mycotoxins in the animal feed industry in Mali.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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