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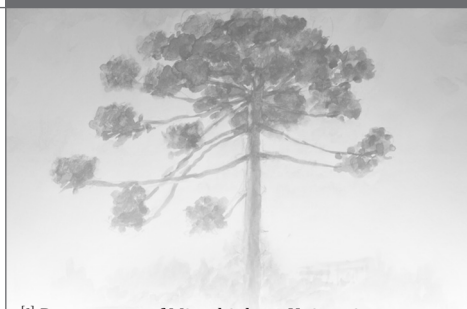

Micro-fungi and mycotoxins in poultry dust

Microfungos e micotoxinas em poeira de confinamento

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Abstract

Dust in poultry confinement facilities were investigated for their fungi and mycotoxins contents. Concentrations of microfungi in the air of poultry confinement facilities were determined using air samplers, and fungal isolation from air by plate exposure. Settled dusts were investigated for their fungal load, types of fungi present and mycotoxins present. Microfungal load in settled dust and air of poultry confinements were found to be 3.5-42 x 10^6 cfu/g and 5-119 x10^5 cfu/m^3 respectively. Fungi isolated from poultry confinements were: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus ochraceus Penicillium notatum, Mucor racemosus, Penicillium oxalicum, Trichoderma viride, Stachybotrys atra, Fusarium oxysporum, Candida albicans, Cryptococus neoformans and Saccharomyces cerevisae*. Mycotoxins concentrations obtained from the analyses of sieved poultry dust were 21.32 $\pm$ 2.35 ppb of aflatoxins, 11.26 $\pm$ 1.78 ppb of ochratoxins and 4.10 $\pm$ 0.13 ppb of fumonisins. Aflatoxin concentrations and fungal loads in settled dust showed positive correlation with duration of dust deposition, but the former produced stronger association than the latter ($r = +0.991$, $P < 0.001$ and $r = +0.957$, $P < 0.02$ respectively). **Results** obtained in this study indicate that poultry dust is rich in mirofungi and mycotoxins which could be of occupational health importance.

Keywords: *Air sampling. Mould. Mycotoxins. Occupational health. Poultry dust.*

Resumo

Poeiras presentes em ambientes de confinamentos de aves foram avaliadas para verificação da ocorrência de fungos e micotoxinas. Para tal, foram utilizados amostradores de ar e realizado o isolamento de fungos do ar pela exposição de placas. As poeiras foram investigadas quanto à quantidade e tipos de fungo e micotoxinas. A carda de microfungos quantificada foi de 3,5-42 x 106 ufc/g, 5-119 x105 UFC/m^3, respectivamente, estando presentes as seguintes espécies: Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus ochraceus, Penicillium notatum, Racemosus mucor, Oxalicum penicillium, Trichoderma viride, Atra stachybotrys, Fusarium oxysporum, Albicans candida, e Cryptococus neoformans e Saccharomyces cerevisiae. *As concentrações de micotoxinas obtidas nas análises de pó peneirado foram de 21,32 2,35 ppb de aflatoxinas, 11,26 1,78 ppb de ocratoxinas 0,13 e 4,10 ppb de fumonisinas. As concentrações de aflatoxina de fungos e cargas em pó apresentaram correlação positiva com o tempo de deposição de pó, mas o primeiro produzindo associação mais forte do que o último ($r = 0,991$, $p < 0,001$ e $r = 0,957$, $p < 0,02$, respectivamente). Os resultados obtidos neste estudo indicam que a poeira presente em ambientes de confinamento de aves de capoeira é rica em microfungos e micotoxinas que possam ser de importância para a saúde ocupacional.*

Palavras-chave: *Avaliação da qualidade do ar. Mofo. Micotoxinas. Saúde ocupacional. Poeira de confinamento.*

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Introduction

Fungi affect human and animal well beings in variety of ways, such as diseases of essential crop plants, decay of stored foods with possible concomitant production of mycotoxins, superficial and systemic infections of both human and animal tissues. The spores of a large number of important fungi are less than 5 μm-aerodynamic diameters and are therefore able to enter the lungs. They may also contain significant amount of mycotoxis. Diseases associated with inhalation of fungal spores include toxic pneumonitis, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure and cancer (1). With exception of mushroom toxins, approximately 350-400 fungal metabolites are considered to be toxic (1). The most important mycotoxins in agriculture are the aflatoxins, ochratoxins, the 12,13-epoxytrichothecenes and the fumonisins; which are produced by species of fungi belonging to the genera *Aspergillus, Penicillum* and *Fusarium.* Other toxigenic fungi include *Stachybotris atra* and the species of *Alternaria, Paecilomyces, Trichoderma* and *Trichothecium*. All of these fungi occur commonly in soil, agriculture products, grain dust and house dust (2).

Species of fungi in which mycotoxins have been reported in the spores include *Alternaria alternata* (3), *Aspergillus fumigatus* (4); *Aspergillus flavus* and *Aspergillus parasiticus* (5), *Fusarium graminearum* (6); *Fusarium sporotrichioides* (6) and *Stachybotris atra* (1). It was suggested that workers and others who handle infected grains may be at risk of exposure by inhalation. Since majority of mycotoxins are non-volatile, mycotoxin exposure by inhalation is most likely to occur via inhalation of spores. (1).

Mycotoxins are a diverse group of fungal secondary metabolites, which are generally harmful to animals and humans. Mycotoxicoses are diseases that result from consumption of or exposure to mycotoxins (7).

A mycotoxin contaminated diet may lead to substantial economic loses in livestock due to feed refusal, poor feed conversion, diminished body weight gain, immune-suppression, interference with reproductive capabilities and residues in animal products (8). Harmful effects of various mycotoxins to animals and humans have been well documented by Jacobsen et al. (7).

The mycotoxins producing fungi may contaminate agricultural products in the field (pre-harvest), during storage (post-harvest) or during processing (8).

Mycotoxins are currently considered as a serious threat to the poultry farming in terms of diseases leading to synergistic interactions with other infections. Mycotoxins that have been associated with poultry feeds/ingredients contamination include: aflatoxins, ochratoxins, trichothecenes, citrinin, streigmatocystin and diacetoxysarphenol (9). T-2 toxins have several effects on poultry, causing oral lessons and immuno-deppression. The fumonisins can affect the growth performances of broiler at doses as low as 75 ppm (10). Stoev et al. (11) reported a significant decrease in body mass and relative weight of lymphoid organs of broiler chicken fed a mouldy diet containing Ochratoxin and penicillic acid. They equally observe pathomorphological changes such as cloudy swelling and granular degeneration in the epithelium and mononuclear cell infiltration and activation of capillary endothelium in the kidney and liver; degenerative changes and depletion of lymphoid organs (bursa of Fabricius, thymus and spleen).

Exposure to microfungi-laden dust in poultry housings can result in a variety of disease conditions. Mycopathy is a collective term used for diseases caused by fungi, either living or dead or their metabolic products (toxins, allergens or enzymes). This complex term comprises disease manifestations e.g. mycosis, mycotic colonisation, myco-allergosis, mycetism and mycotoxicoses (9).

Material and methods

Concentration of fungi in air

This was carried out with the aid of personal samplers as described by Hendric et al. (12). Air was drawn through isopore filters (ATTD 0.8 μm, Millipore, Cambridge Mass, USA) with the aid of a pump at a flow rate of 5 L/mm for 30 minutes. The filters were rinsed in sterile water. The aliquot (0.1 ml) was plated on nutrient agar for bacterial load on one hand and potato dextrose agar from fungal load on the other hand.

Isolation of microfungi from air in poultry confinements

Sterile plates of potato dextrose agar, PDA and subouraud dextrose agar, SDA (Oxoid, England) incorporated with penicillin and streptomycin were carefully

exposed to air for 2 minutes in poultry confinements. Following exposure, the lids were replaced taken to the laboratory and incubated for 3 – 5 days at room temperature (28±2 °C). Discrete colonies were sub-cultured, fungi isolated and characterized based on their cultural and morphological appearances.

Isolation of fungi from settled dusts and poultry litter

The 10^4 and 10^3 dilutions of poultry dust and litters were found best for fungal growth. One millilitre of each of the two dilutions was mixed separately with 9 ml of PDA and SDA (Oxoid, England) enriched with penicillin (250 units/mL) and streptomycin (0.05 g/mL) in sterile plates. The plates in duplicates were incubated at 28±2°C for 3 – 5 days. The fungal colonies that developed were counted and sub-cultured to obtain pure cultures, which were identified according to Barnet and Hunter (13) and Domsch and Gams (14).

Mycotoxins assay in poultry dusts

Investigations were carried out for the presence of aflatoxin, ochratoxins and fumonisins in settled poultry dusts, by methods described by AOAC (15).

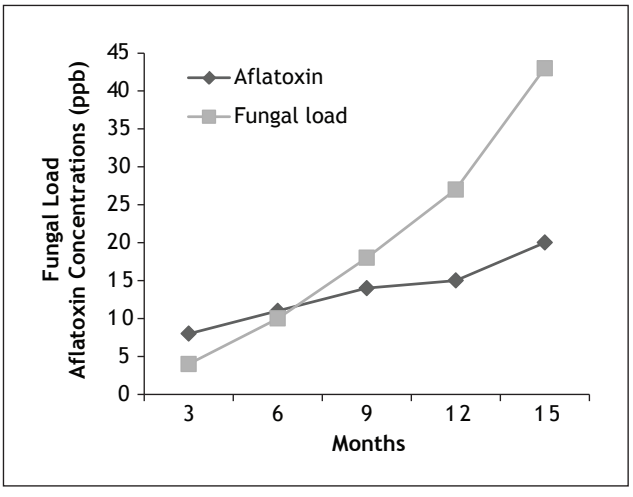
Aflatoxin and ochratoxin tests by enzyme linked immunosorbent assay (ELISA): The concentration of aflatoxin in compounded feed samples were determined by a direct competitive enzyme-linked immunosorbent assay (ELISA), using the Agra Quant® Total Aflatoxin Assay 4/40 Kits (Romer Laboratory Inc., Singapore). Twenty grammes of ground feed samples were added to 100 mL of 70% methanol for extraction of aflatoxin and filtered. The filtrate and enzyme conjugated aflatoxin were mixed and added to antibody coated microwell. Aflatoxin in samples and standards were allowed to compete separately with enzyme conjugated aflatoxin for antibody binding sites. After a step of 5 washes, an enzyme substrate was added and blue colour developed. This was followed by addition of stop solution. Absorbances were read at 460 nm by a computerized microplate reader and total aflatoxin expressed in parts per billion (ppb). The concentration of ochratoxin in each feed sample was determined using AgraQuant® Ochratoxin Assay Kits (Romer Laboratory Inc., Singapore). The technique is also a direct competitive ELISA. The procedure was same as described for aflatoxin assay.

Aflatoxin and fumonisins assay by high-performance liquid chromatography: High-performance liquid chromatography, HPLC, was used for determination of the concentrations of aflatoxins (B_1, B_2, G_1, G_2) and total fumonisins in poultry dust. Methanol-water extraction of the sieved poultry dust was analysed in the HPLC system coupled with UV, a diode array detector.

Results

Mycotoxins concentrations obtained from the analyses of sieved poultry dust were 21.32±2.35 ppb of aflatoxins, 11.26±1.78 ppb of ochratoxins and 4.10±0.13ppb of fumonisins (Table 1). In a study carried out over a period of one year, in a deep litter poultry pen, aflatoxin concentrations and fungal loads in settled dust on the walls of the pens showed positive correlation with duration of dust deposition, but the former produced stronger association than the latter ($r = +0.991$, $P < 0.001$ and $r = +0.957$, $P < 0.02$ respectively; Graphic 1).

Microfungal load in settled dust and air of poultry confinements were found to be 3.5-42 x 10^6 cfu/g and 5-119 x 10^5 cfu/m^3 respectively. Fungi isolated from poultry confinements were: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus ochraceus, Penicillium notatum,*



Graphic 1 - Variation of aflatoxin concentration and fungal load in poultry dust over a period of 15 months in a poultry building (Aflatoxin: r = +0.990, p = 0.001; Fungal load: r = +0.983, P = 0.003; Fungi/Aflatoxin: F = 27.245, r = +0.949, p = 0.014)

Source: Research data.

Mucor racemosus, Penicillium oxalicum, Trichoderma viride, Stachybotrys atra, Fusarium oxysporum, Candida albicans, Cryptococus neoformans and Saccharomyces cerevisae (Table 2). Mycological investigation of poultry litter over a period of 10 weeks, from the day fresh wood shavings were introduced, showed a positive correlation for fungal load (r = +0.972, P = 0.006) as well as variation in fungi species with age of litter (Table 3).

Table 1 - Mycotoxins in poultry dust

Mycotoxins (ppb)	Confinements average	Rearing house of birds < 8 wks.	Deep litter housing adult birds	Battery cage system
*Aflatoxin B1	10.99 ± 0.35 (9.34-15.31)	16.30 ± 1.29	11.80 ± 1.27	12.72 ± 2.14
*Aflatoxin B2	4.13 ± 0.22 (2.98-5.27)	4.57 ± 0.99	3.69 ± 0.51	4.02 ± 0.64
*Aflatoxin G1	5.52 ± 1.92 (2.18-9.24)	7.20 ± 1.88	2.82 ± 0.88	4.59 ± 1.60
**Total Aflatoxin	20.64 ± 1.42 (14.64-28.41)	28.07 ± 0.49	18.31 ± 2.52	21.33 ± 2.35
*Fumonisins	2.83 ± 0.92 (2.18-6.75)	6.48 ± 0.38	3.29 ± 1.93	4.10 ± 0.13
**Ochratoxins	12.71 ± 0.75 (5.21-17.73)	15.64 ± 2.12	8.16 ± 1.42	11.26 ± 0.13

Values are mean ± SD; Range in bracket; Aflatoxin G2 was not detected;

* Analyzed by HPLC

**Analyzed by ELISA

Source: Research data.

Table 2 - Frequency of isolation of fungi in poultry confinements

(continues)

Fungi	Frequency of occurrence (%)		
	Litter (25)	Settled dust (50)	Airborne (50)
Aspergillus flavus	72	68	52
Aspergillus niger	60	56	44
Aspergillus fumigatus	32	20	18
Aspergillus ochraceus	16	20	12

Table 2 - Frequency of isolation of fungi in poultry confinements

(conclusion)

Fungi	Frequency of occurrence (%)		
	Litter (25)	Settled dust (50)	Airborne (50)
Penicillium notatum	42	38	16
Penicillium oxalicium	28	22	6
Mucor racemosus	78	72	66
Alternaria alternata	12	4	-
Fusarium oxysporum	10	6	-
Trichoderma viridis	16	10	6
Stachybotrys atra	8	4	-
Saccharomyces cerevisae	44	32	24
Cryptococcus neoformans	26	8	-
Candida albican	56	46	16

Sample size in bracket

Source: Research data.

Table 3 - Microfungi in poultry litter monitored over a period of 10 weeks in a deep litter pen

Specimen	Fungal load (x10^5 cfu/g)	Isolated fungi
Fresh wood shaving	1.8	*Penicillium notatum, P. oxalicum.*
1 week poultry litter	2.0	*Penicillium notatum, P. oxalicum, Aspergillus niger*
4 week litter	3.4	*Penicillium notatum, P. oxalicum,* Aspergillus niger, A. flavus Mucor racemosus
10 week litter	3.7	*Penicillium notatum, P. Oxalicum,* Apergillus niger, A. Flavus, Mucor racemosus, Trichoderma viride, Crypyococcus neoformans *Saccharomyces cerevisae*

r = +0.972, P = 0.006

Source: Research data.

Discussion

High fungal load was obtained in both settled dust and air of poultry confinements in this study. Similar high fungal load in poultry air has been reported in Germany (16). Fourteen different species of fungi were isolate. The health consequence of occupational exposure ranges from development of mycosis to mycotoxicosis.

The isolated fungi in this study are referred to as opportunistic fungi. They do not usually induce diseases, but do so when the body host defence is compromised (17). Among the important diseases associated with human exposure to organic dust is allergic broncho-pulmonary aspergillosis (ABPA), which occurs in patients with atopic asthma whose respiratory tracts are colonized with *Aspergillus* species. Clinical findings of ABPA include wheezing and generalized rales. The chest x-ray may demonstrate recurrent migratory infilterates or evidence of central bronchiectasis and mucus plug impaction. Patients with chronic diseases may also demonstrate evidence of pulmonary fibrosis on chest x-rays (17, 18).

Mucor species and other Zygomycetes can invade the tissues of immuno-compromised individuals such as persons suffering from diabetes mellitus, severe burns, leukaemia, hymphoma, or other chronic diseases and immune-suppression. The fungi can invade and proliferate in the walls of blood vessels thereby producing thrombosis. This often occurs in the paranasal sinuses, the lungs and the gastrointestinal tract resulting in ischemic necrosis of surrounding tissue with massive infilteration of polymorphonuclear cells. Such conditions are referred to as mycormycosis when produced by *Mucor* species and zygomycosis when caused by Zygomycetes in general (17).

Exposure to fungi, particulary *Stachybotrys atra* (*Stachybotrys chatarum*) has been linked to cases of idiopathic pulmonary haemorhage (IPH) among infants (19). Between the year 1993 and 1998, a total of 138 cases of IPH were reported in the US (20). The incidence of pulmonary haemosiderosis in Sweden was found to be 2.4 per 10^7 children per year (21) and a value of 1.1 per 10^6 children per year in Japan (22). *Stachybotris atra* produces potent (trichothecene) that are toxic to both humans and animals. The symptoms produce by *S. atra* among exposed farm workers include nasal and tracheal bleeding, skin irritation and alteration in white blood cells (20).

Increase in fungal load with time in poultry litter and changes in species of fungi content was reported in this study. This variation in fungi species is an indication that there could be enrichment of the litter from time to time through feed, air and fomites.

Reasonable concentrations of aflatoxins, ochratoxins and fumonisins in poultry dust were reported in this study. This is an indication that the microfungi in poultry confinements produce potent toxins, which can cause various deleterious toxicological effects on the health of the birds as well as on the workers when dust is inhaled. Aflatoxins are commonly produced by *Aspergillus flavus* and *A. parasiticus*, as well as other *Aspergillus* species. Ochratoxins are produced by the *Aspergillus ochraceus* group and a number of species of *Penicillium*, especially *P. viridicatum*, while the fumonisins are produced by *Fusarium moniliforme* (7). Since many species of fungi are reported in this study, and such fungi are known to produce different types of toxins, it implies that the poultry dust consists of a variety of mycotoxins. Human exposure to poultry dust could therefore produce a variety of mycotoxicoses.

Aflatoxin was found to show a stronger positive correlation than fungal load with duration of dust deposition. This is because mycotoxins are retained in dead spores and mycelia of fungi. Inhalation of such old dust could hence expose such people to more health risk. This strong correlation of mycotoxin with duration of dust deposition could be exploited, as mycotoxin content of dust in an environment may be used to estimate its age; this may require further studies. Beside the mycotoxins content of dust, with potent toxicological effects, the β-D-glucan content of cell walls of fungi (whether dead or alive) are potent toxicological and inflammatory agents (23).

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