ISOLATION AND IDENTIFICATION OF MYCOTOXIGENIC ORGANISMS IN POULTRY FEED FROM SELECTED LOCATIONS IN ABIA STATE, NIGERIA

ABSTRACT

Feed contamination by fungi can lead to nutrient losses and detrimental effects on animal health and production. This present study was designed to isolate and identify the mycological contamination of poultry feeds in some selected parts of Abia state (farms and feed depots in Umuahia north, Osisioma and its environs). A total of 120 samples were collected and used for the study. The samples were screened and processed using spread plate technique. The isolates were identified using slide culture technique. From the samples collected, the fungi contamination in feed samples from depots in Umuahia was 50%, Osisioma 78% and in farms it was 85%. Five fungi organisms were isolated from the feed sample which includes *Aspergillus, Penicillium, Fusarium, Mucor* and *Yeast* which were seen in almost all the feed samples. *Aspergilllus* (87%) recorded the highest percentage occurrence, followed by *Penicillium* (27%), *Fusarium* (24%), Yeast (5%) and Mucor(2%). The total fungi load was significant at 2.0 × 10^5CFU/g=1 for feed samples from Umuahia North Local government Area, 7× 10^5CFU/g=1 from Osisioma feed depot and 1× 10^6CFU/g=1 from poultry farms thereby making the feed samples unsafe for poultry consumption. Therefore, there is need for screening of feeds in these locations in Abia state due to ts high fungal load and percentage contamination.

Keyword: Feed, mycological agents, identification, occurrence, location

Introduction

The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes and nutritional quality (Cegielska-Radziejewska et al.,2013). Moulds like other microorganisms will assimilate and utilize the most readily available nutrient in the material they grow upon and spoilage may lead to the loss of some of the nutrients in the feed (Okoli et al.,2006).

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Among microorganisms, fungi have important effects on the quality of feed. Fungi growth sometimes leads to non-consumption of feed for poultry (Magnoli et al., 2007; Magnoli et al., 2005) .Several factors may lead to the spread of fungi infections such as geographical location, storage conditions, processing of various feeds and moisture. Among the mentioned factors, moisture is the most important factor, hence, rendering the moisture in feed constant to lesser percentage will eliminate fungal growth and aflatoxin production will be stopped (Pitt and Hocking, 2009)

Mycotoxins are poisonous toxins/substances and secondary metabolites produced by fungi (Tola and Kedebe, 2016; Lereau et al;2012; Monbaliu et al;2010). The filamentous general of fungi produces secondary metabolites which have deleterious effects on human and animal consumers following consumption of contaminated foods and this ultimately affects the economy of the country (Mestafa et al.,2012).

Most toxic species belong to the genera Aspergillus, Penicillum, Fusarium, Alternaira and produce mycotoxins that are of public health importance/concern such as aflatoxin, Ochratoxin A, T2-toxin, fursarotoxin, furmonisins, patulin, zearalenone and deoxynivalenol (Gimemo et al.,2007;Iqbal et al.,2014; Orellano.,2007). Feed contaminated with mycotoxins negatively affect poultry performance and their health (Monson et al., 2014). Most mycotoxicosis of poultry are caused by intake of low concentration of contaminants over a long period of time resulting in the typical chronic symptoms of poor growth, poor feed efficiency and suboptimal production. Ingestion of high 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 (Mabbet, 2004).. Fungi causes a significant loss in the poultry industry being responsible for high morbidity and mortality rate especially in young birds and causes stunted growth and diarrhea and fetal encephalitis (Moss, 1992). They also cause drop in egg production leading to economic losses (Cast,2003). In this work, presence of potentially mycotoxigenic fungi in samples of poultry feed was determined. Abia state is in Nigeria which is a tropical country with a predominant hot humid environment and the environment is much favorable for the propagation of fungi on feed and feed materials. To prevent economic losses in poultry, isolation and identification of birds affected by fungal infection needs to be determined and such

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studies on commercial broiler feed sample in Abia state is not well reported. This informs the essence of this study in Abia state south east Nigeria.

MATERIALS AND METHODS

STUDY AREA

Samples were collected between April – June from 2 local government areas of Abia state. (Umuahia and Osisioma)

Abia state in Nigeria is located in a tropical rainforest between latitude 543N and longitude 752E. The average annual temperature and rainfall are 26.9^0C and 2193mm respectively (Kottek *et al*.,2006).

SAMPLE COLLECTION

Poultry feeds were sampled from farms and different feed depots in 2 different local government areas (Umuahia North and Osisioma). The total feed samples collected were one hundred and twenty (120) in number (which includes Top feeds, Vital, Animal care, and Apex feed) was used to isolate and identify the presence of mycotoxigenic fungi. Forty (40) feed samples were collected from each of the locations. Also 40 feed samples were collected randomly from poultry farms within the 2 localities. The representative samples were collected batch by batch using simple random sampling technique. The sampling plan was carried out according to Food and Agriculture Organization (FAO, 1993). Take 10grams from each batch and mix them together. Samples were collected two weeks intervals and collection lasted three months (April-June). Fungal contamination and fungal count determination was carried out in each sample to determine the fungal genera and the total fungal population in the Department of Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike

Fungal Isolation and identification

Laboratory procedure

Sabouraud dextrose agar medium was used for the isolation of fungi in the feed samples. The medium was prepared aseptically following the manufacturer's description. After autoclaving, a calculated amount of penicillium and streptomycin was mixed with the medium to help inhibit

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the growth of bacteria. Therefore, the medium was dispensed into sterile petri dishes in aseptic environment.

Serial dilution plate technique (Omenka and Anyasor, 2010) was used for fungal isolation and general fungi counts. One gram of each of the representative samples was mixed with 9ml of sterile distilled water on a horizontal position and shake for 30mins to form uniform suspension. For each feed sample, five dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} 10^{-5} were made from each dilution, 0.1ml of dilution was aseptically inoculated on Sabouraud dextrose agar supplemented with penicillin and streptomycin (Vesna et al., 2010). A surface spread plate technique was used to achieve uniform distribution of the spores. Inoculated plates were incubated at 25^0c for 5-7days for isolation of the fungi and overall quantitative enumeration of fungal colonies per gram of the feed sample, isolates were identified based on colonial and microscopic morphologies (Andersen *et al.*,2003; Pitt and Hocking1997). Microscopic examination of the isolate was done using wet mount and slide culture technique (Leck,1999). The relative occurrence of fungal genera was calculated in percentage using the following

$$\text{Percentage occurrence of fungal genus: } \frac{\text{Number of isolates} \times 100}{\text{Total Number of Fungi}}$$

$$\text{Total fungal load CFU/g: } \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume used}}$$

4.1 RESULTS

Plate 1 and Plate 2 shows the morphological presentation of the colonies of *Aspergillus specie* and *Fusarium species* which appears in form of an emulsion as brownish and whitish colouration. Plate 3 show colonies typical of *Penicillium specie* marked with remarkable in-folding while Plate 4 reveals different colouration consistent with colonies of mixed fungi infection.

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Figure1 shows the presence of *Aspergillus spp* at magnification of ×100, the marked evidence of oval dark hypha measuring about 0.5mm can be comparable to similar findings of Aspergillus occurrence. Figure 2 presents a remarkable cauliflower with distinctive dark hypha and unique long conidiosphore characteristic of Aspergillius. Fig3 shows marked long conidiospore with many branches about (6-10) with long dark hypha and diameter of about 0.5mm. Fig 5: indicates aggregates of fungi hypha called mycelium.

From the study, the fungi species isolated and identified down to genus level are *Aspergillus, Penicillium, Fusarium, Yeast and Mucor.*

Table 1 show that *Aspergillus penicillium* and *fusarium* contamination was recorded in the 3 locations, yeast was absent in samples collected from Umuahia while *mucor* was present only in farms. Table2: shows the total samples collected from each location and the positive numbers, 85% were positive from farms, 78% were positive from Osisoma and 50% were positive from Umuahia.

From the above study, the genus aspergillus had the highest frequency of isolate at 85% followed by penicillium (27%), fusarium (25%), yeast (5%) and mucor (@%) as shown in figure 6. Table 3 shows that feed sampled from farms has the highest fungal load followed by samples from Osisioma then samples from Umuahia feed distributors and depot. Table 3: suggests that the feed sampled from poultry farms and Osisioma feed distributors and depots have much fungal load of about $1x10^6$ and $7x10^5$ compared to that from Umuahia which have fungal load of about $2.0x10^5$

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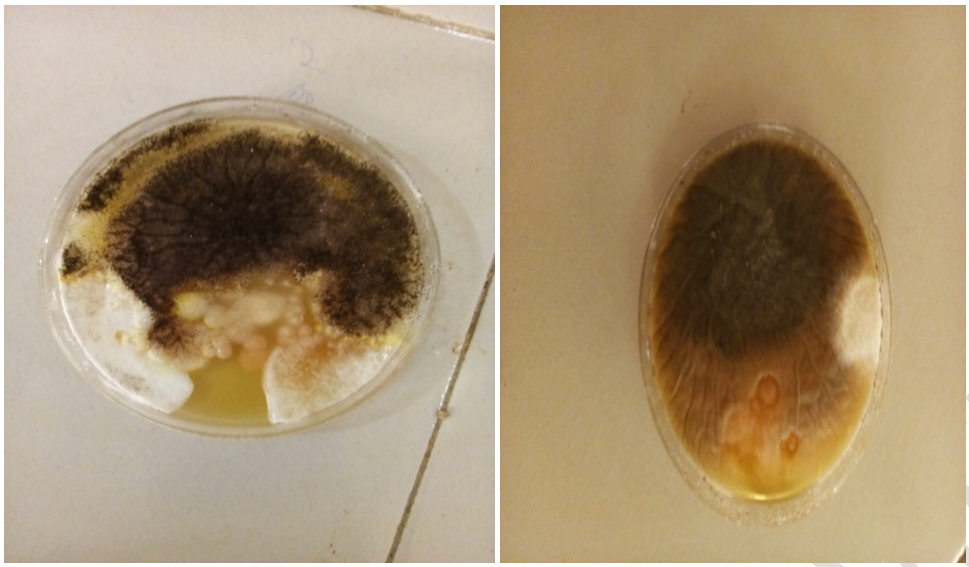

Plate:1 Colonies of *Aspergillus and fusarium*; Plate:2 colonies of *Aspergillus* Morphological view

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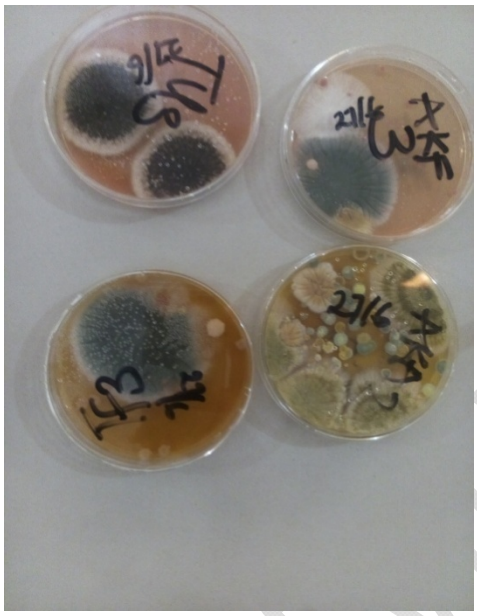

Plate 3: Colonies of *Penicillum spp* Plate 4: colonies of mixed fungi infection (morphological view)

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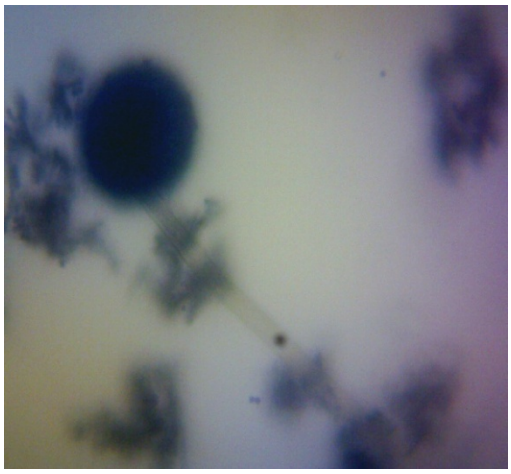
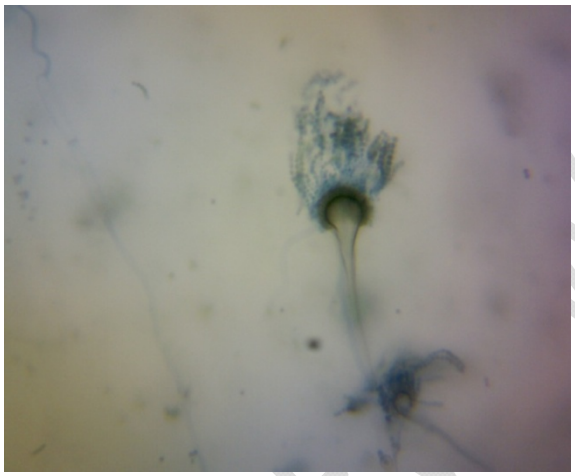

Fig: 1 *Aspergillus* ×100MG Fig 2: *Aspergillus* view ×100MG

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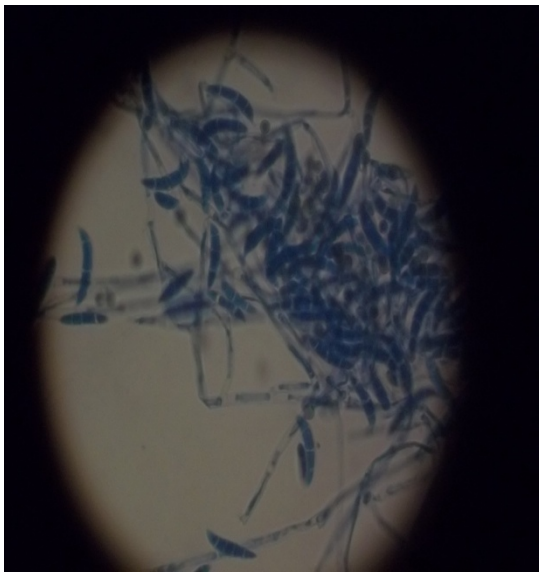

Fig3 *Fusarium spp* x100MG

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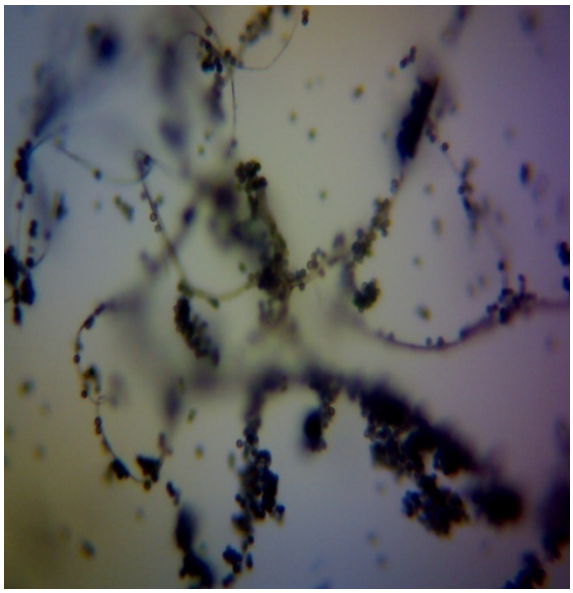

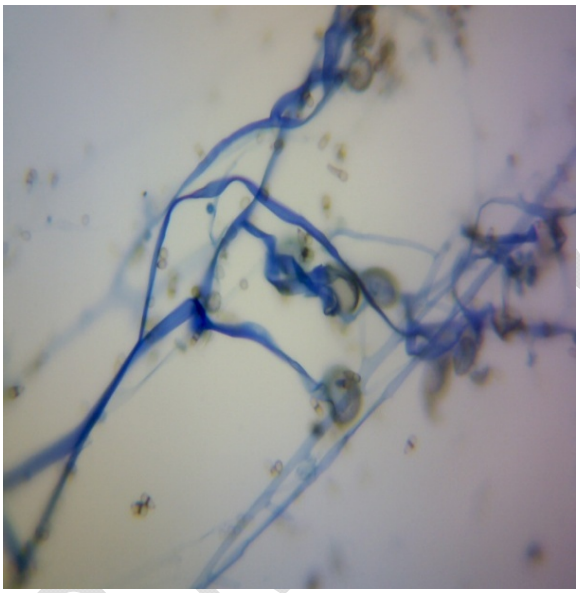

Fig4: *Penicillium spp* x100MG Fig5: *Fungi mycelium* x100MG

Table 1: Fungi Genera Isolated From Some Selected Locations in Abia State

Fungi organisms	Umuahia North	Osisioma	Farms (both)
Aspergillus	+	+	+
Penicillium	+	+	+
Fusarium	+	+	+
Yeast	_	+	+
Mucor	_	_	+

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Keys + (positive) – (Negative)

Table2: Percentage and frequency of Fungi Contamination of The Feed Sampled From Various Locations

	Locations	No of samples	No of samples contamination	Level of % contamination
	Farms	40	34	85
	Osisioma	40	31	78
	Umu North	40	20	50

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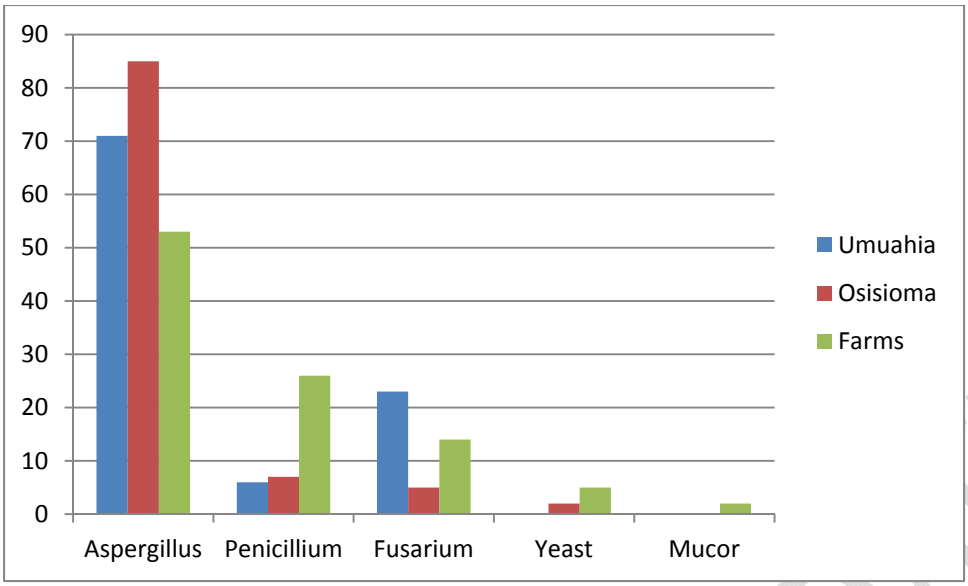

Figure6: Percentage occurrence of Fungi organisms Isolated from 3 different locations in Abia State

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Table 3: Total fungal load of feed sampled from each location

	Locations	Total fungal count CFU/g-1
	Umuahia North	$2.0x10^5$
	Osisioma	$7x10^5$
	Farms	$1x10^6$

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DISCUSSION

The study established that all the poultry feeds sampled harbored one fungi organism or the other. Most of these organisms found in the poultry feed are those commonly found in soil and water. The fungi isolated in this study were similar to those microorganisms reported by (Makun *et al*., 2010; Atehnkeng *et al*., 2008; Kpodo *et al*., 2010). Also from this result there is indication that feeds from farms has the highest percentage of fungal contamination of about 85% (Table 2) and this may be due to poor sanitary measures adopted in the processing and storage or due to poor environmental and personal hygiene practice in the farm as well as lack of proper biosecurity. *Aspergillus s*pecie has the highest fungi percentage occurrence affecting most of the poultry feed sampled and this can be as a result of the organisms ability to thrive in high osmotic pressure and this is in agreement with (Geiser *et al.,* 2009). Also because its spore are common component of aerosols and they drift on air current dispersing themselves both short and long distances and when in contact with solid feeds or liquid surfaces they tend to germinate if the condition of the moisture is right and this view is consistent with the findings of Gioconda and Richard, (2004). From the study, *Aspergillus species* was the predominant organism isolated and this findings is in agreement with (Rosa *et al*.. 2006; Oliveira *et al*., 2006; Figueora *et al*., 2009)

This research could not ascertain whether contamination occurred at the manufacturer level, retailers or farmers, though, (Pitts and Hocking 1997; Monge *et al*., 2013) established that *Aspergillus* is predominant in cereals and other ingredient used in producing poultry feeds in the tropics. Contamination of poultry feeds particularly by pathogen may occur prior to processing, distribution and or storage. Other studies have similarly concluded that cereals and other ingredient use in producing poultry feed may be source of product contamination. This does not exclude the fact that environment/ moist surface facilitate the growth of fungi. The occurrence of *Aspergillus, Penicillium and fusarium spp* could be due to absorption of moisture during storage.(Gow, 2002). The stored poultry feed might have reabsorbed moisture from the environment which then supported the growth of the microorganism in addition to the contamination during processing.

The total fungal load in the analyzed finished feed samples in this study were about $1.9x10^{6}$cfu/g-1 which is higher than that reported in Slovakia, in 2003 of 1.9×10^{3}cfu/g-1) as reported by Magnoli, (1994). According to mycological quality criterion, good fungal count

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should be less than 3×10^4. (Adesokan, 2005) The fungal load of poultry in this study was found to be higher than the required load, hence the sampled poultry feeds are not good for poultry consumption because they could lead to aflatoxicosis which results in reduction of both production rate and meat quality. Also, from this result there is indication that feeds from farms has the highest fungal count of about$1x10^6$ (Table 4) and this may be due to poor sanitary measures adopted in the processing and storage or due to poor environmental and personal hygiene practice in the farm as well as lack of proper biosecurity, followed by feed samples from Osisioma which have about $7x10^5$ which could be as a result of high stocking density. The Feed samples from Umuahia has the least fungal load of about $2.0x10^5$ which may be due to good sanitary measures and low stocking density adopted by feed distributors and depots in Umuahia.

The presence of fungi in the poultry feeds was analyzed using ANOVA of 95% confidence interval and value $p < 0.05$ considered statistically significant. Also the post hoc shows that there was a strong association between the presence of *aspergillus and fusarium, penicillium, yeast and mucor*.

CONCLUSION

Since no vaccine exist for any of the fungal diseases of poultry therefore, the timely adoption of good management practices, strict biosecurity, effective disease diagnosis and suitable preventive measures along with necessary treatment like use of probiotics with appropriate chemotherapeutic agents are good measures to have a check and control the fungal disease of poultry apart from the fungal infection. *Aspergillus, fusarium, penecillium* and *mucor* were the main fungi isolated while *yeast* is a related fungi organism. Mycotoxins are a major concern as they are the leading cause of immune suppression in birds lowering their resistant level in viral and bacterial disease and increase mortality. Thus a holistic approach is required to combat the adverse effect on high economic returns from the poultry production. There is need for regular surveillance and monitoring of important mycotoxins with the use of conventional as well as modern diagnostic.

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310 REFERENCES

311 Cegielska-Radziejewsk R, Stuper K , Szablewski T. “Microflora and mycotoxin contaminations
312 in poultry feed mixtures from western Poland”. Annuals of Agriculture and Environmental
313 medicine. 2013 : 20(1): 30-35

314 Okoli CI, Nweke CU, Okolie CG, Opara MN. “Assessment of the mycoflora of commercial
315 poultry feeds sold in the humid tropical environment of Imo State, Nigeria.”. International
316 Journal of Environmental Science and Technology. 2006: 3: (1):9-14

317 Magnoli C, Astorece A, Chiacchiera SM, Dalcero A. Occurrence of Ochratoxin A and
318 Ochratoxygenic mycoflora in corn and corn based food and feeds in some South American
319 Countries. Mycopathologia. 2007: 163:249-260 (Pub Med) (Google Scholar)

320 Mangoli C, Hallak C, Astoreca A, Ponsone Lo, Chiacchiera SM, Palacio G. Surveillance of
321 toxigenic fungi and ochratoxin A in feedstuff from Cordoba province. Vet Resource
322 Communique. 2005: 29:431-445. (Pub Med)(Google Scholar)

323 Pitt J, Hocking A. Fungi and Food spoilage. 2009 .3rd edition,Springer Berlin-Germany.
324 (Google Scholar)

325 Tola M, Kedebe B. Occurrence, Importance and Control of mycotoxins; A review. Cogent Food
326 and Agriculture, 2016:doi:10.1080/23311932.2016.1191103

327 Lereau, M., Gouas, D., Villar, S., Besaratina, A.,Hantefeuille, A Berthillion, P., Martel-Planche G,
328 Da costa AN, Ortiz-Cuaran S, Hantz O, Pfeifer GP. Interactions between hepatitis B.virus and
329 aflatoxin B_1 Effects of P_{53} induction in Hepa RG cells. Journal General Virology. 2012: 93 (3): 640-
330 650

331 Monbaliu S, Van Poucke C, Detarernier C, Dumoulin F, Van De Velde M, Schoeters E, Van
332 Dqck S, Averkieva O, Van-Peteghem C, De Saeger S. Occurrence of mycotoxins in feed as

UNDER PEER REVIEW

analysed by a multi mycotic LC-MS/MS method. Journal of Agriculture and Food Chemistry. 2010: 58(1):66-71

Mostafa A, Armin A, Hamid P, Reza AM. Review paper: Rapid detection method for analysis of fungi and mycotoxins in Agricultural products. Research Journals of Recent Sciences. 2012: 1(7):90-98

Gimeno A Martins ML. Micotoxinas y micotoxicosis en animales y humanos, Special Nutrients, Miami, Fla, USA, 1st edition 2007

Iqbal SZ, Rabbani T, Asi MR, Jinap S. Assessment of aflatoxins, ochratoxin A and zearalenome in breakfast cereal. Food Chemistry. 2004: 157: 257-262.

Orellano JI. "metodos de determinacion,identificacion y control de micotoxinas en ingredients para la nutricion animal" Engormix, 2007.View at Google Scholar.

Monson MS Settlage RE, Mcmahoon KW, Mendoza KM, Rarwal S, El-Nezami HS, Coulombe RA Reed KM. Response to the hepatic transcriptone to aflatoxin B_1 in domestic turkey (Meleagris gallopavo) PLos ONE 2014: 6: e100930

Mabbett T. "keep feeds free from fungi" African farming. 2004: pp 15-16.View at Google Scholar in green colour.

Moss MO . Mycotoxic fungi. In: Microbial Food Poisoning. In A.R. Elley (Ed), Chapman and Hall, London, Glassgow, New York, Tokyo, Melbourne, Madras. 1992 pp 73-106

Council for Agricultural Science ad Technology (CAST). Mycotoxins: risks in plant, animal and human systems. Task force Repot No 139, Ames, IA 2003

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. World map of the koppen-Griger climate classification updated. Meteorological Zeitschrift. 2006: 15:259-264

Food and Agricultural organization of the United States. Prevention and reduction of food and feed contaminantion. The Codex Alimentarius Commission. !st Edition Rome. 1993.

UNDER PEER REVIEW

Omenka RO, Anyasor GN. Vegetable based feed formulation on poultry meat quality. African Journal of Food agriculture Nutrition and Development. 2010:10 (1): 40127=40132

Vesna SK, Ljiljana SD, Snezana TT. The frequency of pathogenic fungi genera in poultry feed. Journal of Food Agriculture and Environment. 2010: 8 (3): 589-591

Anderson IC, Campbell CD , Prosser JJ. Potential bias of fungi 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environmental Microbiology. 2003: 5 : 36-47

Pitt JJ, Hockings AD. Primary keys and miscelleneonus fungi.In fungi and food spoilage (2nd edi pp 59-171).Blackie academy and professional.Londan. Weinheim, new York, Tokyo, Melbourne,madras. 1997.

Leck A. Preparation of Lactophenol Cotton Blue Slide Mounts Community eye health. AB'S Veterinary Microbiology. 1999:12 (30): 24-25

Makun HA, Anjoriin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Surajundee AA. Fungal and aflatoxin contamination of some human food commodities in Nigeria. African Journals of Food Science. 2010: 4(4): 127-135.

Atehnkeng J, Ojiambo PS, Donner M, Ikotun C, Sikora RA, Cotty PJ. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from agro–ecological zones in Nigeria , international journal of food microbiology. 2008: 122: 74-84

Kpodo K, Thrane U, Hald B. Fusaria and Fumonisins in maize from Ghana and their co-occurrence with aflatoxins. International journal of food microbiology, 2000: 61: 147-157.

Geiser DM, Aoki T, Bacon CW, Baker SE, Bhattacharyya MK, Brandt ME. Letter to the editor one fungus, one name: Defining the genus Fusarium in a scientifically robust way that preserves long standing use. Phytopathology. 2013: 103: 400-408

Gioconda SB, Richard AC. Pathogenic fungi: Host interactions and emerging strategies for control. 2004.

UNDER PEER REVIEW

Rosa CAR, Riberio JMM, Fraga MJ, Gatti M, Cavaglieri LR, Magnoli CE, Dalcero AM, Lopes CWG. Mycoflora of poultry feeds and ochratoxins- producing ability of isolated *Aspergillus* and *Penicillium* species. Veterinary Microbiology. 2006: 113: 89-96.

Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcero AM, Rosa CAR. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Ro de Janeiro state, Brazil Mycopathologia. 2006: 162 (5): 355-362

Figueroa S, Centeno S, Calvo MA. Renggel A, Adelantado E. Mycobiota and concentration of ochratoxins A in concentrated poultry feeds from Venezuela. Parkinstan Journal of Biological Sciences. 2009:12: 589-594.

Monge MP, Dalcero AM, Magnoli CE, Chiacchiera SM. Natural co-occurrence of fungi and mycotoxins in poultry feeds from Entre Rios Food Additives and Contaminants. 2013: 6:168-174

Gow NAR, Brown AJP, Odd FC. Fungal morphogenesis and host invasion. Current Opinion in Microbiology. 2002: 5(4): 366-371.http://.dx. doiorg/10.1007/bf00442768.

Magnoli P, Monge MP, Miazzo RD, Cavalieri LR, Dalcero AM, Chiacchiera SM. Effect of low levels of aflatoxin B1 on performance, biochemical parameters and aflatoxin B1 in broiler liver in the presence of monensin and sodium bentonite. Poultry Science. 1994: 90 (1): 48-58

Adesokan IA, Ogunbanwo ST, Ode loyinbo BB. Microbiological quality of selected brands of beer in Nigeria. In the book of Abstract of the 29th annual conference and general meeting (Abeokuta 2005) on microbes as agent of sustainable development, organised by Nigerian Society of Microbiology (NSM) University of Abeokuta from 6-10th Nov. 2005: pp 21

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