ASSESSMENT OF WATER AND FEED AS SOURCES OF MYCOTIC INFECTION IN CATTLE AND PIG FARMS IN IBADAN, OYO STATE, NIGERIA

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ABSTRACT

Mycotic infections on farms can be attributed to contaminated feed and water. The storage facilities and processes used to keep feedstuffs are largely unhygienic, and contribute to the growth and survival of pathogenic organisms that have impacts on animal health, production and the economy. This study aimed at assessing the presence, type and frequency of fungi in the feed and water served to cattle and pigs in the study areas. A total number of 100 water samples (50 from cattle farms and 50 from pig farms) and 100 feed samples (50 feedstuff from cattle and 50 feed stuff from pigs) were collected from farms in 8 locations within Ibadan, Oyo State. These include WofunOlodo, Iyana church, Olorunsogo, Ologunerun, Teaching and research farm, University of Ibadan, Akinyele, Barika and Iyana Church. Samples were transported in ice pack into the laboratory within 5 hours. Samples were cultured using Sabaroud dextrose agar (Oxoid, UK) at 25^0C, 37^0C and analysed for fungal growth. From the pig feed samples, 29 (48.4%) plates were positive for fungi growth while 14 (34.12%) plates of water samples yielded fungi growth. From the cattle feed samples, 38 (62.70%) plates were positive for fungi growth while 14 (35%) plates of water samples yielded fungi growth. The isolated species of fungi from the water and feed samples were *Aspergillus, Mucor, Penicillium, Rhizopus and Absidia species.* This finding shows that feed and water contain fungi which can be a source of infection to pig and poses threat to the public health.

Keywords: Pig, Cattle, Feed, water, and Fungi

Introduction

Beef is an important agricultural commodity in the world economy (Smith *et al*,. 2018). Generally, world beef production constitutes about 40 percent of the livestock output (FAO, 2005). Nigeria is by far the leading beef cattle producer in Central and West Africa (Al-Mustapha *et al*., 2025). Nigeria's cattle herds are estimated at over 16 million heads, far ahead of Niger (8.7 million), Mali (8.2 million) and Chad (7 million) (Grain de Sel, 2010). The beef cattle sub-sector plays important roles in the Nigerian economy, not only in terms of its contribution to the gross domestic products (GDP) but also contributes substantially to the supply of animal protein (Tewe, 2010).. Pigs are omnivores and are highly social and intelligent animal (Angier, 2009). Pig, which is among the source of animal protein in Nigeria are monogastric animal with a high rate of productivity and has high ability to utilize a wide range of agro-industrial waste products, crop residue and household waste with little or no processing and at minimal cost. (Tewe & Adesehinwa, 1995; Igwe *et al*., 2014) and therefore have the potential to boost the nation's animal protein supply. But despite the potential of pig to bridge the large supply gap in animal protein supply, several factors mitigate against pig production, which include

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growth of Islamic fundamentalism that oppose pig production (Umeh *et al*., 2015), inadequate extension service, pest and diseases, lack of credit facilities, low productive feed, problem of pig marketing, price fluctuation, housing and management problem and other problems like lack of water, high cost of feeding and transportation (Folasade *et al*., 2011) Pork is one of the most popular forms of meat for human consumption, accounting for 38% of worldwide meat production (Agarwal, 2023). Animal feed industry is an integral and growing segment of the food supply chain in Nigeria. It supplies the necessary feed ingredients to produce healthy animals which provide food protein and energy that are essential for human beings. Feeding cost accounts for 50-85% cost of commercial pig production depending on the level of intensification (Osondu *et al*,. 2014). A fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms (Tortora, *et al*,. 1995).The FAO reported that about 25% fungal contamination of feed raw materials and finished feed causes infectious diseases in the flock, or worse still induce secondary infections (Kpodo *et al*., 2000). In addition, handling contaminated animal feed can also raise health issues regarding workers exposure to fungi and mycotoxins (Viegas *et al*., 2016) It has been demonstrated that the presence of microscopic fungi affects the quality of feeds, their organoleptic attributes, and nutritional quality (Cegielska-Radziejewska, 2013) Molds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrients in the feed (Okoli *et al*,. 2006). Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic toxic effects. With respect to humans and animals, mycotoxins exhibit toxic actions and are characterized by carcinogenic, mutagenic, teratogenic, and estrogenic properties (Cegielska-Radziejewska, 2013). This study aims at assessing the presence of fungi in the feed and water served to cattle and pigsand also to determine the types and frequency of the fungi isolated from the feed samples in the study areas.

Experimental

Materials and methods

Study areas

The study was conducted in 4 local government areas of Ibadan, Oyo state, Nigeria. These are Akinyele, Ibadan-North, Ido and Lagelu Local government areas.

Sample size

A total of 200 samples were collected for the study which include 100 water samples and 100 feed samples.

Ethical issues

Ethical Approval was collected from Animal Care and Use Committee (ACUREC) of the University of Ibadan with the number: UI-/20/043ACUREC and UI-ACUREC/20/045

Sample collection

A total of 200 samples (100 water and 100 feed samples: 50 feedstuffs from cattle and 50 feed stuffs from pigs) were collected from farms in Ibadan, Oyo State. Cattle and pig feedstuffs and water samples were collected from their feeding troughs, watering points and storage points at the farms. Feedstuffs collected include cassava peelings, guinea corn, and concentrates fed to the animals. Samples were put in ice pack and transported to Microbiology laboratory, University College Hospital (UCH), Ibadan.

Fungi isolation procedures

A volume of 15 ml of the water sample was

collected into each sterile sample bottles. The bottle was shaken slightly and 10mls of the water sample was collected into plastic test tubes and covered. The water was then centrifuged at 2000rpm for 10 minutes. After the centrifugation, the supernatant was decanted to leave about 0.5ml in the test tube. From this, 2 loopful of the wire loop was inoculated on SDA and incubated at room temperature (25^0C) and 37^0C. A total of 1 gram of the feed samples was measured into sterile test tubes with an electronic weighing balance. Sterile distilled water was added to it to make up to 10mls mark of the test tube. It was left to stay for 30 minutes and subsequently vortexed. From the supernatant, 2 loopful was inoculated onto SDA and incubated at room temperature (25^0C) and 37^0C.

For each sample, two plates were inoculated, a plate was incubated at 37^0C while the other was incubated at room temperature. The plates were observed every other day and read at 7-10 days. The media used for the study was Sabouraud Dextrose Agar impregnated with chloramphenicol(50 mg/ml) and gentamycin (50mg/ml) (Yousefi, *et al*., 2013). Fungi identification was done using colonial morphology and microscopic characteristics.

Results

Fungi isolated from feed samples

Out of the 60 plates inoculated with cattle feed samples, 62.7% of the plates showed positive fungal growth while 36.7% of the samples were negative. The pathogenic fungi genera identified from the positive plates were *Rhizopus, Mucor, Aspergillus, Penicillium* and *Absidia* while 6.67% of the plates contained non-pathogenic fungi species. *Rhizopus* species were recovered from 11.7% of the plates, *Mucor* from 13.4% of the plates. *Aspergillus* was identified on 18.3% of the plates. *Penicillium* was isolated from 8.3% while *Absidia* was identified on 5% of the plates. (Table 1).

Out of 60 pig feed samples inoculated 48.4%plates were positive for fungi growth. Isolated and identified fungi genera include *Rhizopus, Mucor, Aspergillus Absidia* and some environmental nonpathogenic fungi. *Rhizopus* has the highest frequency of occurrence and was recovered from 13.4% of plates. *Mucor* and *Aspergillus* were both recovered from 10%. *Absidia* has the least frequency of 3.3% of the plates while environmental non - pathogenic fungi were recovered from 11.67% of the plates. (Table 1). Figure 1 shows fungi growth.



Fig.1: Image showing fungi growth

TABLE 1

Fungi isolated from cattle and pigs feed samples

Fungi From cattle feed	Frequency	Percentage (%)	Fungi from pig feed	Frequency	Percentage
Mucor	8	13.40	*Mucor*	6	10.00
Aspergillus	11	18.30	*Aspergillus*	6	10.00
Penicillium	5	8.30	-		
Rhizopus	7	11.70	*Rhizopus*	8	13.40
Absidia	3	5.00	*Absidia*	2	3.33
Non-pathogenic	4	6.67	**Non - pathogenic**	7	11.67
Total	38	62.70	**Total**	29	48.40

Fungi isolated from water samples

Out of 40 plates inoculated with cattle water samples for fungi culture, identification and isolation, 35% of the plates showed positive fungal growth while 65% were negative. The pathogenic fungi genera identified from the positive plates were *Rhizopus, Mucor, Aspergillus* and *Penicillium.* 5% of the plates contained non-pathogenic fungi species.

Rhizopus was isolated from 7.5% of the plates. *Mucor* was found on 7.5% of the plates. *Aspergillus* was isolated from 10% of the plates while *Penicillium* was isolated from 5% of the plates (Table 2). A total of 41 plates were inoculated with water samples of which 34.12% of the plates were positive while 65.88% of the plates were negative. The fungi genera isolated include: *Rhizopus* from 4.8% of the plates, Mucor from 4.8% of the plates, *Aspergillus* and *Penicillium* from 7.3% of the plates each and environmental non- pathogenic fungi from 9.76% of the plates. (Table 2).

TABLE 2

Fungi isolated from Cattle and Pig water samples

Fungi from cattle water samples	Frequency	Percentage (%)	Fungi from pig water samples	Frequency	Percentage %
Mucor	3	7.50	***Mucor***	2	4.87
Aspergillus	4	10.00	***Aspergillus***	3	7.31
Penicillium	2	5.00	***Penicillium***	3	7.31
Rhizopus	3	7.50	***Rhizopus***	2	4.87
Non-pathogenic	2	5.00	**Non-pathogenic**	4	9.76
Total	14	35.00		14	34.12

Discussions

In this study, Aspergillus species, followed by Rhizopus, Mucor, Penicillium and Absidia species were the main fungal genera isolated from the cow feeds and water sampled, whereas Rhizopus has the highest frequency in pig feed and water sampled followed by Aspergillus, Mucor and Penicillium. The high percentage of *Aspergillus* isolated in this study is not surprising because research has revealed that the species have been isolated with increased frequency as causative agents of diseases (Lass-florl *et al*., 2005, Krishnan *et al*., 2009 and Steinbach *et al*., 2012). Fungi of the genus *Aspergillus* are well-known to produce aflatoxins (B1, B2, G1, and G2). These mycotoxins are known to be the most toxic, hepatocarcinogenic natural products (Bennett

& Klich, 2013). *Aspergillus* and *Penicillium* are universal fungi, species of both genera are found almost everywhere on earth. *Penicillium* is dominant in cooler climates, especially the cool temperate zones, while *Aspergillus* is more common in the tropics. (Pitt, 1994). Infections due to *Aspergillus* species result in significant morbidity and mortality. Most infections are attributed to *Aspergillus fumigatus* followed by *Aspergillus flavus* and *Aspergillus terreus* (Person *et al.,* 2010) *A. niger* has been associated with otomycosis (Araiza *et al*., 2006), cutaneous infections (Loudon *et al*., 1996) and pulmonary disease (Ashby *et al*., 2023). *Aspergillus fumigatus* is responsible for invasive aspergillosis, a life threatening disease that usually only occurs in the immunocompromised patients (Fredj *et al*., 2005).

Mycotoxins are toxic secondary metabolites produced by fungi that grow naturally in several agricultural commodities, causing a wide range of toxic effects in vertebrates (Whitlow *et al*., 2010). Organisms of the genus *Penicillium* are known to be of varying degree of mycotoxigenicity. Research work carried out by Girisham and Reddy (2016) revealed 8 different mycotoxins with the Ochratoxin A (OTA) as the predominating mycotoxin. This work reported 5% isolation of *Penicillium* in cattle water and feed while in one survey in Mexico City, Penicillium formed 14% of the spores collected (Pitt, 1994). Results of *Penicillium* recovered from this work is also lower than that of Marianne in 2000 who isolated 38% from animal feed. *Penicillium roqueforti*, which can grow on organic acids has been reported to be the dominant fungus in most silage samples (Whitlow, 2010). PR toxin produced by *P. roqueforti*, are believed to be involved in disease symptoms observed in farm animals, i.e extensive paralysis of cows (Häggblom, 1990), bovine abortion and placental retention (Still *et al*., 1972).

Contrary to this study, two studies of Krnjaja *et al*., (2008) carried out on fungi-contaminated feed in Serbia revealed that *Fusarium*, *Rhizopus* and *Mucor* have been common fungal genera. Depending on environmental factors, most of the cattle feeds have the potential for being contaminated with certain fungi and subsequently mycotoxins. Contamination of feed and the raw materials with mould fungi and mycotoxins can occur during the pre- and the post-harvest periods respectively by field fungi and storage fungi (Magan & Olsen, 2004). *Rhizopus* are toxigenic and able to produce aflatoxins (Aliyu *et al*., 2016). This work reported 11%, , 13%, 7.5% and 4.8%, Rhizopus isolation from cattle feed, pig feed, cattle water, and pig water samples respectively. A very low percentage of *Absidia* was reported in this study. All these organisms have great significant influences on the health of cattle.

In the study areas, fungi contamination in feed and water was found to be present in fairly high proportion. The contamination of agricultural commodities with fungi able to produce toxic metabolites is a worldwide concern. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production have been linked to moldy contaminated foods and feeds (Pardo *et al*., 2005). Recovered fungi genera in this study include *Aspergillus, Mucor, Rhizopus, Penicillium and Absidia.* Apart from the ability of this fungi to induce mycotic infection, they have been reported to produce secondary metabolite known as mycotoxin (D'Mello, 2001). According to Kanora and Maes, (2009), they reported that the three main genera of mycotoxin-producing pathogenic fungi that are important for sow reproduction are *Aspergillus* spp., *Fusarium* spp. and *Claviceps* spp. Of these three genera, not the fungi themselves, but the mycotoxins they

produce are pathogenic for the pig. According to Lawlor and lynch (2001), 25% of the global crop supply is contaminated with mycotoxins. From the point of view of animal production, there are five important classes of mycotoxins, i.e., trichothecene zearalenone, ochratoxins, aflatoxins and fumonisins (Kanora & Maes, 2009). The presence of toxin-producing fungi in feed or raw material does not automatically imply toxicity depending on amount of toxins produced by the fungi population. Each feedstuff can be infected with more than one fungus and each of them can produce several mycotoxins, providing they are mycotoxin producing fungi. Consequently, it is common that many mycotoxins occur simultaneously in feeds (Koshinsky & Khachatourians, 1992). This combination can cause more adverse effects than a single mycotoxin due to additive or synergistic interaction (Kanora & Maes, 2009).

Other fungi that were isolated but with a relatively low frequency are *Penicillium, Absidia* and *Mucor*. All these organisms have great significant influences on the health of the animal. The organisms could cause either localized or systemic infection in immunocompromised animal (Pereira *et al*., 2010). There could also be an outbreak if the organisms are consumed in an infective dose (Webster & weber, 2007)

Conclusions

The presence and growth of fungal propagules in food and feed (spoilage) is a matter of great concern, because this spoilage may be pursued by difficulties such as allergy, human and animal infection and mycotoxin production. Depending on environmental factors, most of the foods and feeds have the potential for being contaminated with certain fungi and subsequently mycotoxins. High occurrence of fungal species along the food chain is of great public health concern. Fungal contamination of animal feed, with the consequent mycotoxin production, is one of the major threats to human and animal health.

This study established the presence of different fungal agents in cow feed and water. This is probably associated with feed storage as well as sources of water served to the animals. The methods of transporting feeds by farmers are also potential factors encouraging mycotic growth. The detection of toxigenic fungi in feed indicate that mycotoxins may possibly be present in the feed, it therefore suggests potential risk of contamination.

Furthermore, since the species isolated are pathogenic to humans too, exposure of humans to these mycotic agents in sufficient doses could cause disease in healthy individuals. This report underlines the need for proper feed storage and availability of treated water for cattle. The feed should not also be stored for long periods of time.

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