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Mycotoxins Contamination in Animal Food and Feed

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Abstract: ***Many fungi, both field and storage fungi produce metabolites that are poisonous, sometimes fatal, to men and animals. Production of these mycotoxins depends on species or strain of the fungus and on the ecological conditions for its development, in particular food source, moisture, temperature, mechanical damage, hot spots, time duration, O_2 concentration, CO_2 level, nature of substrate mineral nutrition, chemical treatment, plant stress, invertebrate vectors, fungal inoculums, fungal strain differences and interaction of different microorganisms. Grains those are spoiled and unfit for human consumption or having poor quality are generally used for making animal food and feed. There are now over 400 recognized mycotoxins found in feed material and it has been reported that as much 25% of the world's cereal grains may be contaminated with mycotoxins. Mycotoxins are toxic, chemically diverse secondary substances or metabolites produced by wide range of fungi including Aspergillus, Penicillium, Fusarium, Trichothecium, Claviceps sps. etc. recognition of the symptoms of mycotoxins poisoning in an affected animal can be quite difficult to do confidently because many of the symptoms of chronic but sub acute cases are the same as those resulting from a wide range of causes. Common symptoms include: rough coat, poor body condition, low milk yield with poor quality milk components, foot lesions, poor reproductive performance, an increase incidence of abortion and inconsistent much with mucus tags. In present investigation 8 different compositions such as khali containing alsi; khali (mustard); chuni(arhar dal); chuni (urad dal), rice, wheat and gram bran mixture; hay and fine rice chaffs were collected from various storage conditions and animal feed centre and were tested for mycotoxin contamination. Isolation of associated fungi was made in Czapak Dox Agar, Malt Extract Agar and Potato Dextrose Agar media. More than 53 isolates belonging to 15 different genera were islolated and are : Aspergillus flavus, A. candidus, A. terreus, A. glacus, A. fischeri, A. nidulans, A. niger, Absisia coryambifera, Alteranaria alternate, Cladosporium sp., Fusarium sp., Humicola sp., Mucor pusillus, Rhizopus sp. and Trichohecium roseum. Mycotoxin contamination recorded on the basis of the fungi as listed by Jelinek et al (1989). At about 70% samples were found with positive mycotoxin contamination.***

Keywords: Mycotoxins, Animal food and feed, Aspergillus, Penicillium

1. Introduction

Animal feed is any agricultural foodstuff used specifically to feed domesticated livestock such as cattle, goats, sheep, horses, chickens and pigs. Cattle feed is a concentrate, basically comprising cereals and usually by-products of plants and animal sources and this concentrate is then mixed with green fodder. Due to high feed cost, mixing of stale bread, kitchen and bakery wastes in the feed are in practice. These waste products are usually tainted with fungus and may be a contributing factor in mycotoxin production in cattle feed.

"Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity (D'Mello and Macdonald, 1998)". There are over 100 species of fungi that can infect plants and produce mycotoxins. Molds can infect dairy cattle, especially during stressful periods when they are immune suppressed, causing a disease referred to as a mycosis. The diverse effects precipitated by these compounds are conventionally considered under the generic term "mycotoxicosis", and include distinct syndromes as well as non-specific conditions. Mycotoxins can be formed on crops in the field, during harvest, or during storage, processing, or feeding. Molds are present throughout the environment. Spore concentrations are high in the soil and in plant debris, and lie ready to infect the growing plant in the field.

Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Contamination may also occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi. Moisture content and ambient temperature are key determinants of fungal colonization and mycotoxin production. It is conventional to subdivide toxigenic fungi into "field" (or plant-pathogenic) and "storage" (or saprophytic/spoilage) organisms. Claviceps, Neotyphodium, Fusarium and Alternaria are classical representatives of field fungi while Aspergillus and Penicillium exemplify storage organisms. Mycotoxigenic species may be further distinguished on the basis of geographical prevalence, reflecting specific environmental requirements for growth and secondary metabolism. Thus, Aspergillus flavus, A. parasiticus and A. ochraceus readily proliferate under warm, humid conditions, while Penicillium expansum and P. verrucosum are essentially temperate fungi. Consequently, the Aspergillus mycotoxins predominate in plant products emanating from the tropics and other warm regions, while the Penicillium mycotoxins occur widely in temperate foods, particularly cereal grains. Fusarium fungi are more ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereals from warm countries. Mycotoxins can be the primary agent causing acute health or production problems in a dairy herd, but more likely, mycotoxins are a factor contributing to chronic problems

including a higher incidence of disease, poor reproductive performance or suboptimal milk production.

2. Materials and Method

2.1 Sampling: Sample was collected using given methods (Shukla,2015)

Collection of fodder samples: Various fodder samples khali(alsi –Linum asitatissimum) , khali(mustard – Brassica juncea & B.nigra), rice bran (Oryza sativa), wheat bran(choker Triticum aestivum), chuni containing pulses powder such as arahar dal (Cajanus cajan), chuni containing urad dal (Vigna mungo), chuni containing masoor dal (Lens culinaris) and fine rice chaffs (Oryza sativa)] were collected from various storage conditions and animal feed centers with the help of sterilized spatula in sterilized transparent polythene bags. The bags containing sample were sealed and brought to the laboratory for further use.

Sample storage: Collected samples were stored at 4^{o}C in refrigerator, to provide low temperature, low moisture content, low relative humidity storage conditions until they were analysed.

2.2 Analysis: Analysis was done under following:

Isolation of fungi associated with different cattle feeds: small amount of sample was inoculated on prepoured plates of Malt Extract Agar medium plates and Czapek's Dox Agar medium plates. Antibiotics like penicillin and streptomycin were added in medium @30 µg/l to prevent bacterial growth.

Incubation**:** Inoculated plates were kept in incubator at 28^{o} C and 45^{o} C for 72 to 96 hrs.

Identification of microorganisms: for identification of microorganisms fungal smear was prepared and was observed under research microscope and identification was done with the help of available literature and monograph. (Shukla, 1991; Shukla, 2014 d,e)

2.3 Analysis of Mycotoxin: Mycotoxin contamination in various foods is a major threat to health of exposed people. Therefore, mycotoxin analysis is an important subject of many investigations. For the study, following procedure was followed.

Fungi isolated from feed samples namely Aspergillus flavus, A. paraciticus, A. fischerii, A. candidus , A. terreus, A. glacus, A. fischeri, A. nidulans, A. niger, T. roseum and Fusarium culmorum directly inoculated on conical flask containing 150 ml Malt Extract broth medium.

Incubation: Inoculated flasks were kept for incubation in BOD incubator at temperature of 28^{o} C for 7 days.7 days later Fungal mat with thick mycelial growth was appeared in the flask.

2.4 Procedure for calculation of dried weight of fungal mycelium

Whatman filter paper number 42 was kept in hot air oven at 105^{o} C for 24hrs.After 24hrs, the weight of filter paper was taken using an electronic balance.bFungal mat was filtered from the medium using sterilized pre weighed Whatman filter paper and funnel and filterate was preserved for further use. Filtered mycelium was compressed for removal of extra liquid. Thereafter, filter paper containing mycelium was dried in hot air oven and the dried mycelium mass was weighed using an electronic balance (Shukla, 2014e).

Dry weight of mycelium= (weight of filter paper containing mycelium – weight of filter paper)

2.5 Extraction of mycotoxin

The filtrate obtained previously was measured using measuring cylinder and in equal proportion to it ethyl acetate was taken. Both the filtrate and ethyl acetate was taken in a separator funnel and was shaken well upto 30 minutes. Kept for standing for 1 hr and two separate layers was observed. Using the separator funnel upper layer was separated and preserved which conatained ethyl acetate and mycotoxin and lower layer was discarded.

Crude mycotoxin preparation: In waterbath mycotoxin containing solution was boiled at the boiling point of ethyl acetate. The solution was reduced up to 1-2 ml. The obtained solution is the crude mycotoxin. To analyzed the presence of mycotoxin using thin layer chromatography to separate the toxins present in the crude mixture previously obtained.

Firstly, to the TLC plates silica gel mixture (silica gel & water) was spread like a thin film of approx 5mm thickness and was air dried. Thus the solid phase was prepared. (Xie,2009 ; Stahl, 1953 and Shukla 1991)

Crude mycotoxins sample was loaded on the TLC plate using a capillary tube.

Solvent system for toxin determination contained chloroform and methanol (95:5 v/w) this solvent was put into the TLC chamber and then sample loaded TLC plates were kept into the chamber, so that the mobile phase can run over solid phase separating out the toxins.

After 2 hrs the plates were taken out of the solvent system and were air dried.

The air dried plates were observed under the UV chamber for the presence of toxins. If toxin was present in the sample then the bands of toxin was observed under the uv chamber.

3. Results and Discussion

Cattle feed are a complete nutrient diet for cattles having all the source of nutrients such as protein, carbohydrate and fats. On the analysis of animal feed sample in culture

medium a wide variety of fungi were detected. In our investigation we have taken 8 samples namely [khali(alsi), khali(mustard), rice bran, wheat bran(chokar), chuni containing arahar dal, chuni containing urad dal, chuni containing masoor dal and fine rice chaffs] were tested for fungal contamination.

Table 1: Occurrence of fungi in various samples of animal food (Shukla, 2015; Shukla, 2014c)

Name of Microorganisms	*Khali (alsi)*	*Khali (musterd)*	*Chuni (arahar dal)*	*Chuni (urad dal)*	*Rice Bran*	*Wheat Bran*	*Chuni (masoor dal)*	*Fine rice chaff*
Aspergillus flavus	+	-	+	+	-	+	+	+
A. candidus	-	-	-	-	+	+	+	-
A. paraciticus	-	+	+	-	-	+	+	+
A. fiscerii	+	-	-	-	-	+	-	-
A. fumigates	+	+	+	-	+	-	+	+
A. glacus	-	-	-	-	-	+	+	-
A. nidulans	-	+	+	-	+	+	+	-
A. terreus	-	-	-	+	+	+	+	-
F. culmorum	-	-	+	-	-	+	+	+
R rhizopodiformis	+	+	+	+	+	+	-	-
R. oryzae	+	-	-	-	+	+	+	+
Mucor pusillus	-	+	-	-	-	+	+	-
Alternaria alternate	+	+	-	-	+	+	+	-
Cladosprium	+	+	+	+	+	-	-	+
T. roseum	-	-	-	-	+	+	+	-
H. terricola	-	-	+	+	+	+	-	+

We found that the common fungi which occurred in the feed samples were Aspergillus flavus, Aspergillus paraciticus, Aspergillus candidus, Aspergillus fischeri, A. nidulans, A. glocus, A.terreus, Hmicola sp., Trichothecium roseum Fusarium culmorum and Rhizopus species. Rice bran and wheat bran had high fungal contamination whereas Khali (alsi), Khali mustered & Fine Rice chaff had only five fungi are appeared and showed lowest fungal diversity (Table 1).

It is generally found that animal feeds are highly contaminated with mycotoxins due to poor handling and poor storage conditions. Identified species of fungi (Aspergillus flavus, Aspergillus paraciticus, Aspergillus candidus, Aspergillus fischeri, Aspergillus glocus, Aspergillus ocheracius, Aspergillus nidulans, Aspergillus terreus Trichothecium roseum, and Fusarium culmorum) were further inoculated for mycotoxin production in Malt extract broth. After 7 days of incubation the dry weight of fungal mycelium was noted (Table 2).

Table 2: Mycotoxin production by fungi isolated from animal food

S. No	Name of microorgsnism	Wet weight of the mycelium (gm) A	Total water content (gm) B	Dry mycelium weight (gm) (A-B =C)	Mycotoxin production*
1	A. flavus	1.32	0.960	0.360	+++
2	A. paraciticus	1.21	0.962	0.248	++
3	A. candidus	1.20	0.964	0.236	+++
4	A. fischeri	1.28	0.961	0.319	-
5	A. glocus	1.23	0.963	0.267	++
6	A. ocheracius	1.17	0.964	0.206	++
7	A. nidulans	1.44	0.967	0.473	++
8	A. terreous	1.28	0.966	0.314	++
9	F. culmorum	1.14	0.963	0.177	-
10	T. roseum	1.26	0.965	0.295	+

*indicates the visual intensity of spots on TLC plates such as +++strong,++moderate, +poor production of mycotoxin and -indicates no mycotoxins production.

Detection of mycotoxin presence was done with the help of TLC. Mycotoxin producers gave fluorescent bands when observed under UV lamp of Wavelength 365nm.

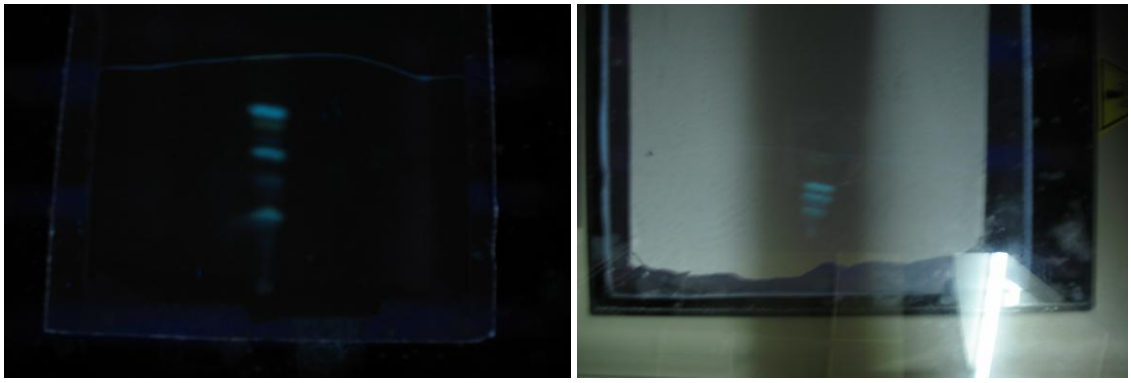

Figure 1: A. flavus & A. paraciticus showing bands of toxin when viewed under UV light

On the basis of visual intensity of bands, the level of mycotoxin was detected. It was found that A. candidus and Aspergillus flavus produced the highest level of mycotoxin and Trichothecium roseum produced the lowest whereas Fusarium culmorum and Aspergillus fischeri shows no production. Also it was found that about 70% of the feed samples were found with positive mycotoxin contamination. Hence, the amount of contamination level is high which will cause the adverse effects to animals.

4. Conclusions

Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes. Animal feeds may also contain endogenous toxins arising principally from specific primary and secondary substances produced by fodder plants. Thus, feed toxins include compounds of both plant and microbial origin. The FAO has estimated that worldwide about 25% of crops are affected annually with mycotoxins. Such surveys reveal sufficiently high occurrences and concentrations of mycotoxins to suggest that mycotoxins are a constant concern. Every year a significant percentage of the world's grain and oilseed crops is contaminated with hazardous mycotoxins such as aflatoxins. Detection, removal and diversion are reasonable means for preventing the entry of mycotoxins into the food chains. The best way of controlling mycotoxin contamination is by prevention and can be accomplished by reducing fungal infection in growing crops through the adoption of suitable cultural practices, by rapid drying or by the use of suitable preservatives (Sinha, 1993) If contamination cannot be prevented, a way to either remove or destroy the toxin will allow consumption of the commodities with reduced adverse effect. Mycotoxins can be eliminated or detoxified by physical, chemical or biological techniques. Many chemicals including numerous acids, alkalis, aldehydes, oxidizing agents and several gases have been tested for their ability to degrade or inactivate aflatoxin and many other mycotoxins. There is need to adopt effective strategies for mycotoxin decontamination and mycotoxin detoxification. The formulation and implementation of mycotoxins regulatory limits, regular analysis of animal feed and feed ingredients and employment of proper mycotoxin decontamination and deactivation strategy will help to reduce the economic losses to a great extent (Shukla,2015).

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