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.

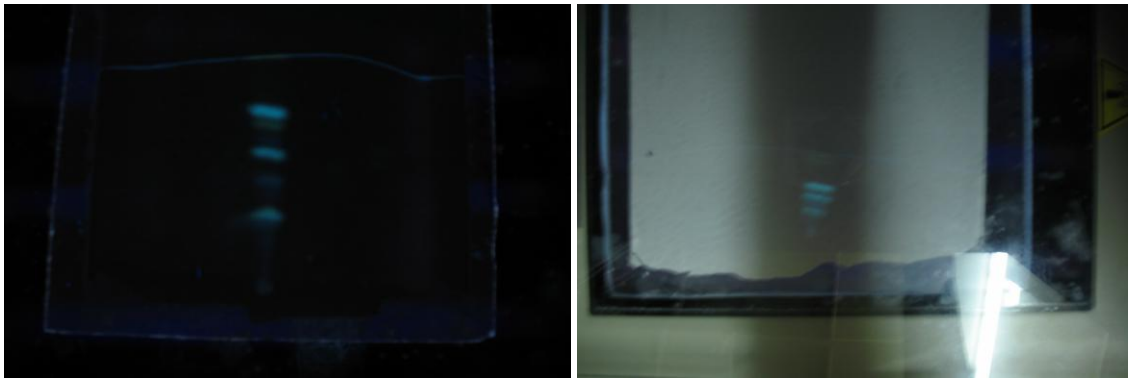

Figure 1: A. flavus & A. paraciticus showing bands of toxin when viewed under UV light

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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