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ASSESMENT OF AFLATOXIN TOXICITY IN CATTLE FEEDS ANA MILK PRODUCTS

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Abstract- Aflatoxins are a group of closely related heterocyclic compounds produced predominantly bytwo filamentous fungiAspergillus flavus and Aspergillus parasiticus.They contaminatevast array of food, animal feed, milk products and agricultural items. Aflatoxin B1, B2 incattle feed and aflatoxin M1 in milk and milk products is considered to pose certain hygienic riskfor human health. The liver is the target organ for toxic effects of aflatoxin B1 (AFB1) and as aresult the metabolism of carbohydrate, lipid and protein in the liver is seriously impaired byAFB1. Reported literature indicates that the acute toxicity results in high fever, jaundice.vomiting, portal hypertension and high mortality rate while chronic toxicity shows humanhepatic cell carcinoma(HCC), AFB1has been implicated in causing a structural DNA alterationand genomic mutation. The aim of our study was to investigate the concentration of aflatoxin B1,B2 in cattle feed and milk products. According to FDA (Food and Drug Administration) actionlevel of aflatoxin in cattle feed in20 ppb. Different samples (makai powder, ready mixed feed, masoor,khalli) and milk products (panner, butter, peda, dahi etc) were analysed using TLCmethod and BGYF (BlueGreen Yellow Florescence test). On the basis of experimental results, it was observed that makai powdercontains aflatoxin B1 with concentration of 0.024 µg/ml and aflatoxin B2 with concentration of0.073µg/ml. The mixed feed contains aflatoxin B1 with concentration of 0.24µg/ml and aflatoxinB2 with concentration of 0.012 µg/ml. The milk product paneer contains aflatoxin B1 in theconcentration of 0.41 µg/ml. It was inferred that cattle feed contains significant amount ofaflatoxin B1 and B2 which may be very harmful for the mammalian system particularly thecattle's which are feeding on it. We also found that among all milk and milk products paneershowed maximum concentration of aflatoxin B1. Since paneer is very frequently used by humans such contaminated food should be consumed with caution. According to FDA (Foodand Drug Administration) the aflatoxin level is 20 ppb in different cattle feeds.

Key words: Aflatoxin, Fungi, Hepatic cell Carcinoma, cattle, BGYF, TLC.

I.INTRODUCTION

Aflatoxins are a group of closely related heterocyclic compounds produced predominantly by afilamentous fungus, *Aspergillus flavus* and *Aspergillus parasiticus*. Recent studies have shownthat *Aspergillus nominus* and *Aspergillus tamarii* strains are also aflatoxin producing of which*Aspergillus nominus* is phenotypically similar to *Aspergillus flavus* [1,2].They can terminate a vast array of substrate and under a variety ofenvironmental conditions. Therefore, most foods are susceptible to aflatoxigenic fungi at somestage of production, processing, transportation and storage.The outbreak of aflatoxicosis (famous as turkey X disease) in England in 1960 caused the deathof a larger population of livestock [3] and led to the discovery of Aflatoxin in groundnut meal and different types of cattle feeds contaminated by *Aspergillus flavus.*Subsequently, aflatoxins were found in other feed especially maize [4] and Cottonseed meal [5]Aflatoxin M (AFM) in milk and milk products is considered to pose certain hygienic risks tohuman health. Mammals that ingest aflatoxin Bcontaminated diets eliminates into milkamounts of the principal 4-hydroxylated metabolite known as " Milk Toxin" or Aflatoxin M. Theeconomic impacts attributed to Aflatoxin are incurred directly by loss in crops, livestock anddairy indirectly by a recurring expenditure in quality control programmes, research and education. Aflatoxin B1 (AFB1) is metabolized by hepatic microsomal mixed function oxidase system, but it can also undergo several metabolic conversations depending upon species [6].The amount of

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Aflatoxin M(AFM) excreted in milk as a percentage of AFB, average 1-2%, varying from animal, from day to day and one milking to other. The AFM could be detected in milk 12-24h after the first AFB ingestion reaching a high level a few days. When theintake of AFB is finished, the AFM and the concentration in the milk decreases to anundetectable level after 72 h [7,8] observed that therewas a linear relationship between AFB1dcse and AFMI into ewes' milk. The internationalagency for research on cancer [9] classified AFB1 and AFM1 as class 1 and 2B humancarcinogen, respectively [10] observed a high genotoxic activity of AFM1,although it was lower than that of AFB1.The Aflatoxin show both acute and chronic toxicity. It seems that milk has greatest demonstratepotential for including Aflatoxin residues from edible animal tissues into human diet. Aflatoxinsare one of the major etiological factors in the development of hepatocellular carcinoma[11] and morerecently associations between childhood Aflatoxin exposure and both growthfaltering[12] have been reported. The occurrence of AFM1 in commerciallyavailable milk products is one of the most serious problem of food hygiene.

II. MATERIAL AND METHOD

2.1 COLLECTION OF SAMPLE

Five feed samples mixed feed, maize powder, masoor, wheat powder and khalli were collectedrandomly for the estimation of Aflatoxin from different cattle feed shops. The cattle feedpowdersamples 250gm of each sample (mixed feed, maize powder, masoor, wheat powder and khalli)was collected in sterilized polythene bags for the estimation of aflatoxin. Five milk and milkproduct sample(milk, dahi, paneer, butter and peda) were collected randomly for theestimation of aflatoxin from local dairy. The milk and milk product samples were collected andweight of each sample was taken in dry Petridis.

2.2 ESTIMATION OF AFLATOXIN FROM CATTLE FEEDS

Extraction of aflatoxin from cattle feed sample was done as per the method. 25gm of all thecattle feed samples were taken and made into powdery form by using mixer-grinder. To each ofthe sample was added methanol and water in the ratio of 6:4 and 62:5 ml to prepare. Themixture was then filtered and the filterate was taken in a separating funnel where 25ml n-hexane and 15ml saturated NaCl was added to the filterate and the mixture was shakenvigorously and left to stand for 30 minutes. The separated layer was taken and mixed with 25mlof chloroform in another separating funnel after vigorously shaking for 30 minutes. Theseparated layer was taken and passed through 250mg $CaCo_3$ and 250mg of Na_2CO_4. Thecollected solution was dried in water bath and 1ml of chloroform was added to the sample andthen applied on silica gel coated TLC plate. This process was repeated for each of the cattle feedsample.

2.3 ESTIMATION OF AFLATOXIN FROM MILK AND MELIK PRODUCTS

Each of the 25ml of milk sample were collected and added to 62.5ml of methanol and distilledwater in the ratio of 6:4 and was mixed properly, filtered and the filterate was collected inother container. 10ml of saturated NaCl and 25ml of n-hexane are added, mixed and shakenvigorously. This step is repeated twice. After half an hour the lower layer is taken and 25ml ofchloroform is added and then taken in another separating funnel. After shaking vigorously andleaving for half an hour the layer was taken and mixed with 250mg of $CuCO_3$ and 250mg of$Na2CO_4$. The collected sample was then dried on water bath and 1ml of chloroform was addedto each of the milk product sample solution. The samples were loaded on TLC plates, theprocess was repeated for all the samples.

2.4 LOAD OF SAMPLE ON TLC PLATES

A thin layer of chromatographic plate was prepared with the help of silica gel and activated inoven. The prepared sample were loaded on TLC plates and run in TIM(toluene, isoamyl alcoholand methanol solution in the ratio of 90:32:3) to separate the aflatoxin according to themolecular weight. A standard sample is also loaded on different space provided in TLC plate.The plate is dried and ninhydrinis sprayed on it. Different spots are obtained which wereinspected under the ultraviolet rays(200 λ). After observation of spots of different aflatoxinspots are scraped for aflatoxin estimation.

2.5 ESTIMATION OF AFLATOXIN FROM TLC PLATES

The spots of mycotoxin on TLC plates are scraped and collected separately. To each of thescraped powder 5ml of methanol was added and mixed properly. The mixture was centrifugedat 3000 rpm for 10 minutes and supernatant was collected and taken in a test tube.Optical density of each sample was taken at 360 nm with the help ofspectrophotometer. A blank tube was prepared for reference in which cold methanol was added.

III. RESULT AND DISCUSSION

Table No-1: Optical density of different type of cattle feed, and milk products were obtained:

SAMPLE SOLUTION	OPTICAL DENSITY
Maize Powder B1	00.001
Maize Powder B2	00.003
Ready Mixed B1	00.010
Ready Mixed B2	00.005
Paneer	00.017

CALCULATION:Concentration (µg/ml) = $\frac{D \times M \times 1000}{E \times L}$

Where,

D = optical densityM = molecular weight of aflatoxin B1 and B2 E = Absorptivity L = path length

Table No -2 : Concentration of Aflatoxin and Optical Density of different sample solution.

SAMPLE SOLUTION	OPTICAL DENSITY (λ)	CONCENTRATION OF AFLATOXIN (µg/ml)
Maize Powder B1	00.001	0.024
Maize Powder B2	00.003	0.073
Ready Mixed B1	00.010	0.24
Ready Mixed B2	00.005	0.012
Paneer	00.017	0.41

On the basis of result the maize powder contains aflatoxin B1 0.024 µg/mland aflatoxin B2was0.073µg/ml. the ready mixed contains B1 with concentration 0.24 µg/mland aflatoxin B2 was0.012 µg/ml and milk pro paneer

contain aflatoxin B1 0.41 µg/ml. The cattle feed usedfrequently for the cattle show content aflatoxin B1 and B2 which is very harmful for animalsfound among all milk and milk products. Only paneer shows maximum concentration ofaflatoxin B1. Paneer is frequently used by humans which can be very harmful for them.Aflatoxins are very potent compound that cause variety of human and animal health problems,in rare conditions livestock can die by ingesting aflatoxin contaminated feed. Most commonlyaflatoxin reduces the feed efficiency and reproductivity of livestock. It can suppress theimmune system of animals leading to more frequent occurrence of infectious disease.Mycotoxin are also considered unavoidable contaminants in food and feed stuffs becauseagronomical technology has not yet advanced to stage at which pre harvest infections ofsusceptible feed by virus can be eliminated [13]. In United States alone, the economic loss from mycotoxin is estimated to be $932 million [14]. Maize, cattle feed and groundnut continue to be major source of aflatoxin particularly inIndia [15]. Sinha had done survey for three consecutive years in some districts of Biharand revealed heavy infestations of mycotoxin producing fungi with different maize samples [16] reported a viable plate count method for detecting mouldcontamination is used to determine the mycological quality of foods and agriculturalcommodities. A high mould count indicates the possibility of aflatoxin contamination but not aconfirmatory test for the presence of aflatoxin contamination but our results obtained by TLCplate method and VGYF showed cattle feed sample like maize powder, ready mixed contain0.024 µg/ml, 0.073 µg/ml, 0.24 µg/mland 0.012 µg/mlconcentration of aflatoxin [17]. found that one sample out of 10 samples of corn and wheat wascontaminated with aflatoxigenic stains of *Aspergillus flavus* and *Aspergillus parasiticus*.Results obtained in this study show that 3 samples out of 10 samples of cattle feed maizepowder and ready mixed and milk products(paneer) was contaminated with aflatoxin.Similar results were reported by [18.19] who detectedaflatoxin B1 at different percentage in examined samples of feed and feed ingredients atconcentration above and below the aflatoxin concentration. The slight variation inconcentration of aflatoxin B1 contaminating samples may be due to differences in locality fromwhich samples were collected or differences in storage conditions.In the another study it was reported that dairy products marketed in Italy,observed loweraflatoxinoccurrences and contamination level in southern Italy then in central and northernItaly [20,21] observed variable increases of AFM1 content inyoghurt related to the milk.

IV.CONCLUSION

The study revealed a high incidence of aflatoxin contaminatedcattle feed samples used frequently for the cattle which may be harmful to the animal healthand milk product like paneer which shows maximum concentration of aflatoxin B1. Paneer isoften used by humans which may be harmful for them also. Precaution should be taken forproper cattle feed storage and milk products in order to prevent microbiological and chemicalhazards.

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