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PRELMINARY INVESTIGATION OF AFLATOXINS IN DIETARY RATION OF DAIRY COWS IN KHARTOUM STATE, SUDAN

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ABSTRACT: *This is a preliminary investigation of the incidence and levels of aflatoxins in dairy cow ration in Khartoum North locality using HPLC. The survey was based on three level of groundnut cakes concentration (low=16-18, medium=19-24 and high=25-32%). The data indicated that 2 out of 18 samples examined were contaminated with aflatoxins B1 (0.013 and 0.014 ppb), these values were below the maximum acceptable limit for dairy cows feeds (20 ppb) as was stated by FAO (1997). However further examination of 2 samples of groundnut cakes from the farms showing the positive sample, revealed 108.3 and 18.4 ppb for B1 and 71.6 and 12.4 ppb for B2, respectively. The study also suggested a relationship between the levels of groundnut cakes level in the feed ration of the dairy cows and the contamination by aflatoxins, as these positive samples were from feed ration of high level of groundnut cakes concentration. The positive samples were from dairy farms that mixed their own ration using a traditional mill. The study also showed the absence of G1, G2 and B2 in dairy cows feeding in Khartoum North locality. From this study it was concluded that ration formulation with different feedstuff could minimized the aflatoxins health risk for dairy animals, however further research is needed in this field.*

ORIGINAL ARTICLE

Key words: *Aflatoxins, Groundnuts Cakes, Dairy Cows, Contamination*

INTRODUCTION

A major handicap facing wide use of groundnut is its high susceptibility to growth and development of moulds, therefore contamination with mycotoxins, notably aflatoxins. Aflatoxins is a group of toxic metabolites, produced by strains of *Aspergillus flavus* and *A. parasiticus*, which proved to be highly toxic to a wide range of animal species including dairy herd (El-Nazemi et al., 2002). Studies have been conducted on aflatoxins contamination of agricultural products in the Sudan (Ali, 2004; Omer et al., 2004). These studies revealed a wide range of aflatoxins content in groundnut seeds and cakes under variable cultural practices, processing techniques and storage conditions. Some of these products were found to be highly contaminated with aflatoxins to an extent, which would limit their use in human foods or animal feeds (Omer et al., 2004). Milk, eggs and meat products are sometimes contaminated because of the animal consumption of aflatoxins contaminated feed (Martins et al., 2007; Hainaut and Boyle, 2008).

Aflatoxins are a group of mycotoxins, which have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect (Ozay et al., 2008). Afaltoxins are classified into B1, B2, G1 and G2; which metabolized to aflatoxins M1 and M2 (Boudra et al., 2007). Aflatoxins B1 is a potent mutagenic and carcinogenic agent found in numerous agricultural and dairy products consumed by humans (Maridgal-Santillan et al., 2007). Aflatoxins contaminated corn and cotton seed meal in dairy rations have resulted aflatoxin M1 contaminated milk and milk products, including skimmed milk, cheese and yoghurt (Van Eijkeren et al., 2006) and pasteurized milk (Zinedine et al., 2007). This study is designed to declare the incidence of aflatoxins in dairy cattle containing ground nut cakes in Khartoum North.

MATERIALS AND METHODS

Source of samples

Eighteen samples of ration were collected randomly from Khartoum State. Samples collection depended on the percentage of groundnut cake in the ration (low: 16-18, medium: 19-24, and high: 25-32%) of dairy cows in

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some of the dairy farms. The dairy rations with positive samples were further investigated by examination of the groundnut cakes for the levels of aflatoxins.

Source of materials

Methanol, chloroform, acetone, iso-propanol and anhydrous sodium sulfate were all of analytical grade. Standard aflatoxins B1, B2, G1 and G2 were Sigma products.

Laboratory examination of ration sample

High Performance Liquid Chromatography (SHIMADZU, DGU-20A3, PUMP, LC-20AB, OVEN CTO-20AC, RF 10AXL, Fluoresce detector), which was made in Japan, 2005 was used for detection of aflatoxins. The extraction was done as was described in AOAC (1980).

Extraction of sample

Fifty grams of each sample were put in 500 ml blender jar to which 200 ml of methanol: water (80:20) v/v was added. The contents were vigorously mixed for 3 minutes (in high speed blender). The extract was filtered through 24 cm Whatmann No.4, in a glass funnel. The filtrate was collected into 250 ml conical flask and moved to separatory funnel. Then 50 ml of 10% sodium chloride (NaCl) was added into the separatory funnel and 50 ml n-hexane was also added to form slurry. After proper mixing, the mixture was separated and the lower aqueous layer was drained.

The obtained liquid was transferred into another 250 ml separation funnel and 50 ml chloroform was added to extract and drain the organic layer. The mixture was shaken gently for 1 minute after which 25 ml chloroform was also added in order to re-extract. The lower chloroform layer (extract) was collected into 250 ml volumetric flask through a layer of anhydrous sodium sulphate (15 g). The collected chloroform extract was evaporated to almost dryness in a water bath, using anti-bumping granules. This extract was kept in a conical flask at room temperature until used.

Column chromatography cleanup

Two grams of silica gel slurry was put in the chromatography column and then 30 ml ether: hexane (3:1) v/v was added to the wash column and silica gel. This was drained off through the stopcorek. The sides of the column were washed also with 2-3 ml of ether: hexane solvent. After that silica gel was settled fully to the open stopcorek and while it was drained, the granular anhydrous (Na_2SO_4) was added to the top of the column. The stopcorek was closed and 2 ml of the chloroform extract was poured into the column and the beaker was washed with 0.5 ml chloroform, which was added to the column. Following this was the addition of 25 ml benzene: acetic acid (9:1) v/v into 250 ml beaker. Ether: hexane (30 ml) was added, while the stopcorek was fully opened to wash the top of the anhydrous Na_2SO_4 layer. To the eluted aflatoxins, 100 ml of dichloromethane: acetone (90:10) v/v was added and the mixture was transferred to a boiling water bath for evaporation of the solvents. The concentrated toxin was transferred quantitatively to a vial using 0.5 ml pipette.

Derivatization

Derivatization of samples were performed by adding 200 µl hexane and 50 µl Trifluroacetic acid (TFA) were added to extract in vial column and capped. The mixture was shaken vigorously using vortex-Genie 2 for 30 seconds and left to stand for 5 minutes. Then 1.950 ml acetonitrile - water (1:9) v/v was added and the mixture was shaken for 30 seconds. The mixture was allowed to stand for 10 minutes to separate. The lower aqueous layer was collected by automatic pipette and used for High Performance Liquid Chromatography (HPLC). Similarly derivation of working standard mixture was done by taking 50 µl of standards and the solvent was evaporated and 200 µl of hexane and 50 µl Trifluroacetic acid (TFA) were added to column extract. Then 1.95 ml acetonitrile: water (1:9) was added and shacken to mix for 30 seconds. The layer was then separated for 10 minutes and the lower aqueous layer was used for HPLC analysis.

HPLC condition

Column length 20 µl, Fluorescent Detector: 360 nm excitation, 400 nm emission, flow rate= 1 ml/min, Oven Temperature = 20 °C, Injection Volume = 20 µl and Sensitivity= medium.

Liquid chromatography system

Samples were compared with standard peaks (Figure 1 and Figure 2), and the concentration in the samples was calculated by using either peak heights or area. Derivatization was performed because of aflatoxins B1 and G1 in aqueous solvents on chromatogram of standard mixture and samples. There were four peaks G2a (from G1), B2a (from B1), G2 and B2 with apparent retention times of 11, 15, 23 and 33, respectively.

Method of aflatoxins calculation

Aflatoxins concentrations were calculated in µg/kg from the following formula:

Concentration of aflatoxins in sample/ µg/kg:-

$$= \frac{\text{area of sample} \times \text{concentration of standard}}{\text{area of standard} \times \text{weight of sample}}$$

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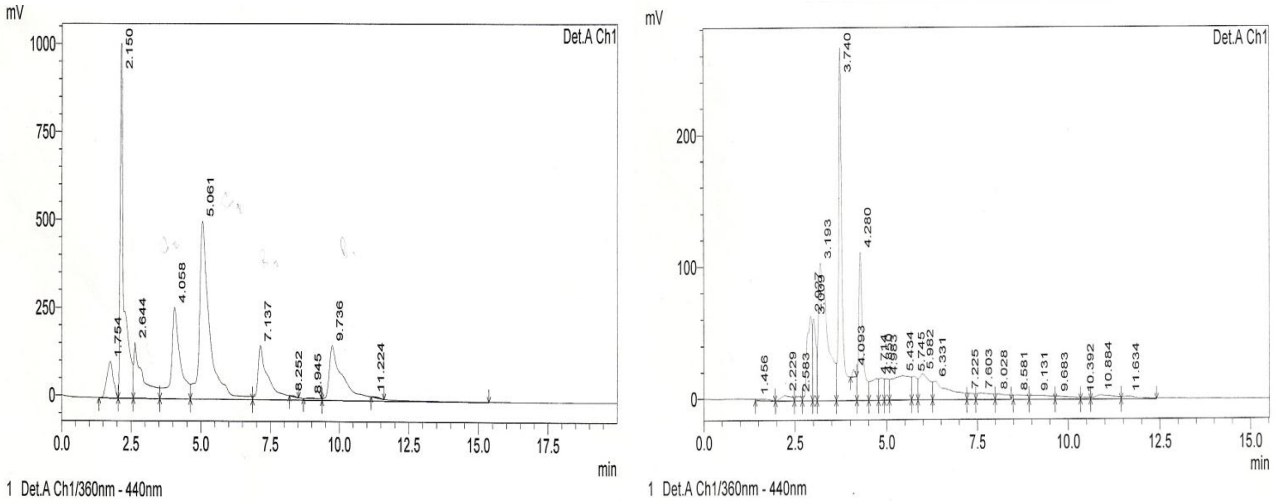
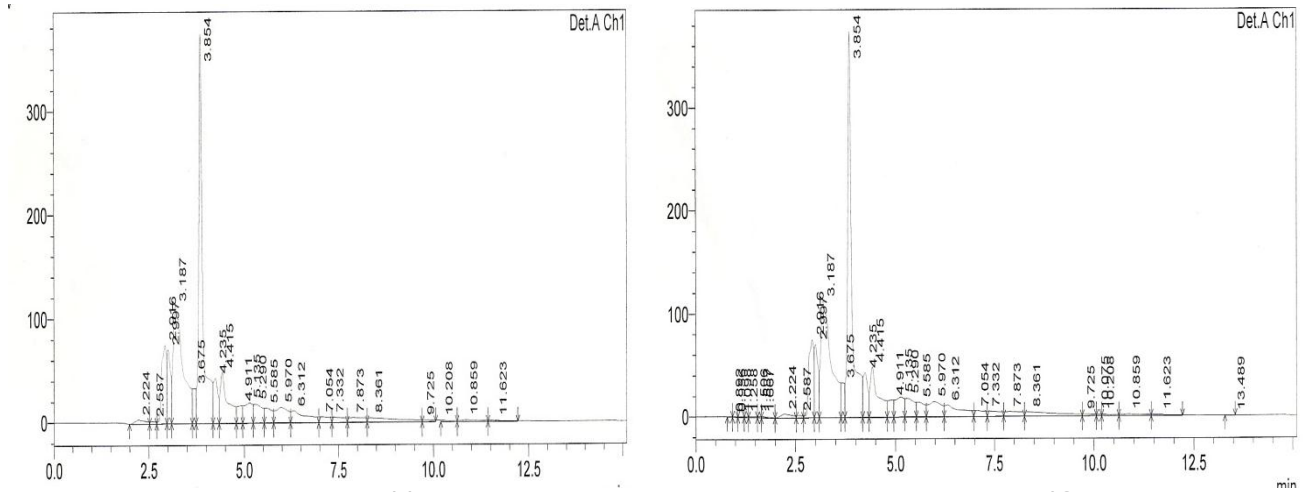
Where:
Concentration of (B1) in standards =0.50 µg/ml
Concentration of (B2) in standards =0.25 µg/ml
Concentration of (G1) in standards =0.50 µg/ml
Concentration of (G1) in standards =0.25 µg/ml



a. Standard b. negative sample

Figure 1 - Aflatoxin peaks of the standard and a negative sample (sample no. 1)



a. sample no. 11 b. sample no. 18

Figure 2 - Aflatoxin detection peak of a positive sample (sample no. 11 and 18)

RESULTS AND DISCUSSION

The present study was conducted in Khartoum North in Khartoum State. The main objective of this study was to evaluate the level and incidence of aflatoxins in the ration of dairy cows. This study showed that aflatoxins B1 was present in the feed of dairy animals, while aflatoxins G1, G2 and B2 were not detected (Table 1). The presence of aflatoxins B1 in the dairy cows ration might create a hazard because the effect of aflatoxins B1 is accumulation (Omer et al., 1998). A linear relationship between the cow's lactation status and feed intake, the daily milk production and aflatoxins B1 concentration in total feed related to aflatoxins M1 level in milk was demonstrated (Van Eijkeren et al., 2006). Moreover aflatoxins B1 is very hazardous to humans and animals as it was regarded as carcinogenic (Omer, 1998; Fardohan and Zoumenou, 2005; Surendranatha Reddy et al., 2011.). Attention should be directed towards the control of aflatoxins especially in cake in dairy cow ration. Many factors were found to affect significantly the incidence of aflatoxins contaminations in groundnuts cake such as type of soil, method of harvesting of crop, method of oil extraction, storage period and the type of store (Ali, 2004). Similarly the moisture content (Omer et al., 2004), the storage conditions (Stephen- Blezinger, 2002; Pazzi et al. 2005), low temperatures (Ghorbanian et al., 2008), relative humidity (Giorni et al., 2007), the season (Tajkarimi, et al., 2007), damaged pods (Ozay et al., 2008) are all been reported as factors. However good agricultural practices durig both pre and post harvest conditions would minimize the problem of contamination by aflatoxins (Stephen-Blezinger, 2002). These include appropriate drying techniques, maintaining proper storage facilities and taking care not to expose grains or oil seeds to moisture durig transport and marketing (Magan and Aldred, 2007).

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Table 1 - Incidence of aflatoxins in the ration of dairy cows in Khartoum North

Percentage of groundnut cake	Sample area	Percentage of groundnut cake in sample	Sample No	Aflatoxins µg/kg			
				G2	G1	B2	B1
Low 16-18%	U of K farm Shabmat	18	1	ND	ND	ND	ND
	U of S farm Hilat KuKu	18	5	ND	ND	ND	ND
	Al-Kadro 1	16	9	ND	ND	ND	ND
	Al-Kadro 2	16	13	ND	ND	ND	ND
	Al-Haj Yosif 1	16	15	ND	ND	ND	ND
	Al-Haj Yosif 2	16	16	ND	ND	ND	ND
Medium 19-24%	Al-Samrab	24	2	ND	ND	ND	ND
	Um doum 1	20	3	ND	ND	ND	ND
	Shambat	24	7	ND	ND	ND	ND
	Al-Droshab	24	8	ND	ND	ND	ND
	Um doum 2	19	10	ND	ND	ND	ND
	Al-ailafon 1	24	12	ND	ND	ND	ND
High 25-32%	Research center Hilat KuKu	25	4	ND	ND	ND	ND
	Al-ailafon 2	28	6	ND	ND	ND	ND
	al-Halfaya 1	32	11	ND	ND	ND	0.013
	Al-Sababi 1	32	17	ND	ND	ND	ND
	Al-Sababi 2	32	14	ND	ND	ND	ND
	al-Halfaya 2	32	18	ND	ND	ND	0.014

ND: Not Detected , Limit of detection 0.1µ/kg

Two out of eighteen samples of the examined feed ration that were analyzed contained aflatoxins B1 (sample 11 and sample 18 which were collected from al-Halfaya area). One of the two farms showing the positive aflatoxins contamination was found to mix their own feed ration using a mill (they store feed ingredients), while the other farm purchase a ready mixed feed ration from a feed mill outside the farm (The further investigation showed that they were from the same source).

The first restore sample No.11 was positive to aflatoxins B1 showed a retention-time of 9.725 and the contamination level of aflatoxins B1 was calculated as 0.014 µg /kg (Figure 1) according to the procedure described in the technical manual. The second positive sample No.18 showed also high retention-time of about 9.725 and when compared with standard it revealed a level of contamination by 0.013 µg/kg. Both positive samples contain percentage of groundnuts cake about 32% to total ration (high level of groundnuts cake). Although the levels were lower compared to the detection limit for feed (20 ppb) stated by FAO (1997), this result is still hazardous because toxin of the accumulated level of aflatoxins (Omer et al., 1998; Fardohan and Zoumenou, 2005). Further investigation, revealed that the original groundnut cakes of sample no. 11 showed 108.3 and 18.4 and sample no. 18 showed 71.6 and 12.4 ppb for B1, B2, respectively (Figure 3). Moreover the detection of aflatoxins B1 in 33.3% of the total feed samples with the high concentration of ground nut cakes (Table 1) indicated that standards and regulation should be adopted in order to minimize level of contamination, because aflatoxins B1 is reported as one of the most potent and potentially lethal metabolite which is well known as human carcinogen (Guzman de Pena, 2007). On the other hand because aflatoxins are very hazardous to animal and human health, young calves are especially susceptible to these toxic effects, which might be largely due to under development of the rumen (Stephen- Blezinger, 2002). It was found that the aflatoxin B1 is directly related with the aflatoxin M1 (Van Eijkeren et al., 2006), B1 in feeds for animal consumption represents a serious problem to human and animal health (Fardohan and Zoumenou, 2005; Van Eijkeren et al., 2006).

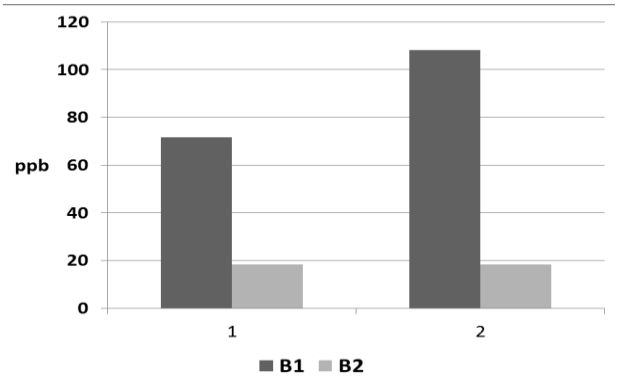

Figure 3 - Detection of Aflatoxin B1 and B2 in groundnut cakes from dairy cows' feed

Mycotoxins attract world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (CTA, 1997). The economic impact of aflatoxins drive directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health (Martins et al., 2007). Control measures include education on the risks of exposure to mycotoxins through skin contact, inhalation and ingestion, early harvesting, rapid appropriate drying, sequestration of diseased seeds from sound seeds, sanitation, use of good agronomic practices, insect control, the use of botanicals and synthetics as storage protectants, biological control and detoxification of mycotoxin-contaminated commodities (Negedu et al., 2011).

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The workshop hosted by World Health Organization to create an integrated plan intended to generate culturally appropriate, long-term, public health strategies to reduce aflatoxins exposure in developing countries (Hainaut and Boyle, 2008). The main recommendation stated clear strategy for afltoxin elimination. The present study support their recommendations include proper handling of crops to prevent mould infection and aflatoxins production in the field and examination or testing of groundnuts cake before addition to dairy cows feed. Also feed storage and distribution should be proper to eliminate the growth of fungus. Education and awareness should be implemented especially among farmers and livestock producers in addition to monitoring programs should be implemented and limits of aflatoxins should be stated for all food and feed. The level of groundnuts cake in dairy cow feeding should not exceed 18%.

The present study concluded that the presence of aflatoxins B in dairy animal feed especially in the high level of groundnut cakes might represents a serious problem of public health in both livestock and animals.

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