

## Determination of Aflatoxins in Animal Feed in Khartoum State, Sudan

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**Abstract:** Aflatoxins are natural toxins that contaminate various types of food and feedstuff leading to health risk in both humans and animals. The aim of this study, was to determine prevalence of aflatoxins contamination and their levels in animal (cattle and poultry) feed samples of groundnut cake meal, wheat bran, crushed sorghum (which are used as raw materials) and manufactured ration in Khartoum State, Sudan. The aflatoxins were extracted using Best Food (BF) method, one of the methods approved by (AOAC), then separated and determined by High Performance Liquid Chromatography (HPLC) adopting Photo Diode Array Detection. The study showed that 64.29% of the samples of animal feed were contaminated by aflatoxins at average concentration of 130.63  $\mu\text{g kg}^{-1}$ . The highest contamination occurred in summer (78.95%) followed by autumn (66.67%) and was found least in winter (47.37%). Manufactured ration showed highest value of contamination (87.50%), with a concentration range of 54.41-579.87  $\mu\text{g kg}^{-1}$  (average concentration of 207.96  $\mu\text{g kg}^{-1}$ ), followed by groundnut cake meal with an overall 69.32% contamination rate and contamination range of 24.29-410.62 (average concentration of 131.45  $\mu\text{g kg}^{-1}$ ), wheat bran with 63.64% contamination rate showed range of 4.07-79.85 (average concentration of 31.19  $\mu\text{g kg}^{-1}$ ) and crushed sorghum with 36.36% contamination rate and contamination range of 5.46-375.81  $\mu\text{g kg}^{-1}$  (average concentration of 165.65  $\mu\text{g kg}^{-1}$ ). Aflatoxin B1 (Afb1) was the most common contaminant followed by Aflatoxin G1 (Afg1), Aflatoxin B2 (Afb2) and lastly Aflatoxin G2 (Afg2).

**Key words:** Aflatoxin, animal feed, groundnut, wheat bran, crushed sorghum, BF method, HPLC

### INTRODUCTION

Aflatoxins are a family of mycotoxins that contaminate peanuts, cereals, cottonseed, corn, rice and other commodities with widespread contamination in hot and humid regions of the world (Murphy *et al.*, 2006). Aflatoxins are extremely toxic, mutagenic and carcinogenic compounds produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Whitlow and Hagler, 2002). Both *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxins Afb<sub>1</sub> and Afb<sub>2</sub> and *Aspergillus parasiticus* also produces aflatoxins Afg<sub>1</sub> and Afg<sub>2</sub> (Santacroce *et al.*, 2008).

Aflatoxin animal exposure causes a variety of symptoms depending on the animal species. However, in all animals, aflatoxins can cause liver damage, decreased reproductive performance, reduced milk or egg production, embryonic death, teratogenicity, tumors and suppressed immune system function, even when, low levels are consumed (Akande *et al.*, 2006). Aflatoxins are both acutely and chronically toxic in animals and humans (Pimpukdee *et al.*, 2004); Afb<sub>1</sub> has been shown through

research to be the most potent naturally occurring carcinogen in animals, with a very strong link to human cancer incidence (Richard *et al.*, 1993; Verma, 2004). The order of potency for both acute and chronic toxicity of aflatoxins is Afb<sub>1</sub> > Afg<sub>1</sub> > Afb<sub>2</sub> > Afg<sub>2</sub> (Santacroce *et al.*, 2008). The USA Food and Drug Administration (FDA) has established 20  $\mu\text{g kg}^{-1}$  as action levels for aflatoxin present in animal feed, this limit is established by the agency to provide an adequate margin of safety to protect human and animal health (Khanafari *et al.*, 2007). The present study aimed to determine prevalence and levels of aflatoxins in animal feeds in Khartoum state where, large populations of dairy cattle and poultry are raised.

### MATERIALS AND METHODS

There are main 4 types of animal feed in Sudan. These feed are: groundnut cake meal (Ombaz), crushed sorghum (Dreesh), wheat bran (Raddah) and manufactured ration. The last mentioned type is prepared in some factories in the area of this study (Khartoum state).

**Samples collection:** Sampling was done by random collection of a total of 56 samples (1-2 kg of weight) of all types of animal feeds were collected during three seasons, namely, summer, autumn and winter. Sites of collection were: factories, sales outlets and farms in Khartoum State. The samples were kept in at  $-20^{\circ}\text{C}$  deep-freezer till tested. At the time of analysis samples were brought up to room temperature (Richard *et al.*, 1993).

**Extraction procedure (BF method):** Feed powder (50 g) was transferred into 500 mL conical flask and 250 mL methanol: water (55: 45), 100 mL hexane and sodium chloride (2 g) were added. The flask was securely stoppered and shaken on a wrist action shaker for 30 min and filtered through filter paper. Total 25 mL of the aqueous methanol lower phase was transferred into a separation funnel. It was then extracted three times with 25 mL chloroform. The combined chloroform extracts were concentrated to 2 mL, approximately. The concentrated extract was then carefully transferred into screw capped borosilicate vial and evaporated to dryness. The dry film was dissolved with 400  $\mu\text{L}$  mobile phase (water: methanol: acetonitrile 60:20:20) and separated by HPLC (Altenkirk *et al.*, 1974; Beg *et al.*, 2006) with use of standard for qualitative and quantitative analysis.

**Separation and detection:** The HPLC-operating conditions were as follows:

- Column type and size: C18; 250 $\times$ 4.6 mm I.D.; 5 micron particle size
- Temperature: Room temperature  $25^{\circ}\text{C}$
- Detector: Photodiode Array  $\lambda$  365 nm
- Mobile phase: Deionized Water: methanol: Acetonitrile (60:20:20)
- Flow rate: 1 mL  $\text{min}^{-1}$
- Injection volume: 20  $\mu\text{L}$

Microsoft Excel was used to calculate averages and coefficient of variations.

## RESULTS AND DISCUSSION

The results of the present investigation revealed high levels of aflatoxin contamination within the samples of animal feed studied. The highest value 207.96  $\mu\text{g kg}^{-1}$  obtained in manufactured ration was remarkably higher than the accepted level of 20  $\mu\text{g kg}^{-1}$  aflatoxins in animal feed (Khanafari *et al.*, 2007).

In manufactured ration samples, aflatoxin was detected in 87.50% of the samples with a concentration range of 54.41-579.87  $\mu\text{g kg}^{-1}$  and an average

concentration of 207.96  $\mu\text{g kg}^{-1}$ . The high prevalence and level of aflatoxin contamination in manufactured rations was not unexpected as these rations are manufactured using ingredients that are stored in bulk and for long times under conditions that favor development of molds.

The percentage contamination of feedstuff varied. In groundnut cake meal samples aflatoxin was detected in 69.23% of the samples in the range of 24.29-410.62  $\mu\text{g kg}^{-1}$  (average concentration of 131.45  $\mu\text{g kg}^{-1}$ ). Whereas, in wheat bran; aflatoxin contamination was found in 63.64% of the samples in the range of 4.07-79.85  $\mu\text{g kg}^{-1}$  (average concentration of 31.19  $\mu\text{g kg}^{-1}$ ). In crushed sorghum samples aflatoxin was detected in 36.36% of the samples with a range of 5.46-375.81  $\mu\text{g kg}^{-1}$  (average concentration of 165.65  $\mu\text{g kg}^{-1}$ ). This variation of percentage contamination may be due to difference in the types of substrates and handling processes from the time of harvest to the time of consumption. Crushed sorghum has shown the lowest percentage contamination, due to the fact that it has not been subjected to man-made negative practices, that is, being easily and instantly prepared in small sale outlets and/or on the farm itself, decrease the time of storage. An earlier study carried out in Sudan showed that aflatoxin was present in 10-100% of groundnut samples with an average concentration of  $8.37\pm 0.61$   $\mu\text{g kg}^{-1}$  (Elamin *et al.*, 1988). Groundnut is known to be more susceptible to aflatoxin contamination (Wild *et al.*, 1993).

It is noteworthy to mention that the level of contamination of wheat bran (31.19  $\mu\text{g kg}^{-1}$ ) obtained in the present study is remarkably higher than that estimated in Kuwait (rang 0-1.07  $\mu\text{g kg}^{-1}$ ) (Beg *et al.*, 2006).

Table 1 shows that AFB1 is uniformly distributed in all feed types. It was detected in (32.14%) of the samples, with an average concentration of 109.68  $\mu\text{g kg}^{-1}$  and concentration range of 5.94-327.73  $\mu\text{g kg}^{-1}$ . It is followed by AfG1 which was detected in 30.36% of the samples, with an average concentration of 108.08  $\mu\text{g kg}^{-1}$  and range of 6.56-556.09  $\mu\text{g kg}^{-1}$ . AFB2 was detected in 28.57% of the samples, with an average concentration of 29.18  $\mu\text{g kg}^{-1}$  and range of 4.07-89.12  $\mu\text{g kg}^{-1}$ . On the other hand, AfG2 was detected in 25.00% of the samples with an average concentration of 30.31  $\mu\text{g kg}^{-1}$  and a range of 1.98-60.49  $\mu\text{g kg}^{-1}$ . The current prevalence of AFB1 percentage contamination reported in the present investigation is higher than that (14.58%) reported in Brazil (Rodriguez-Amaya1 and Sabino, 2002), in which the contamination ranged between 11.5 and 287  $\mu\text{g kg}^{-1}$ , but much less than that (92%) reported in Thailand but with a low average concentration of 7.56  $\mu\text{g kg}^{-1}$  (Charoenpornsook and Kavisarasai, 2006).

**Table 1: Prevalence of different aflatoxin types in animal feed**

Commodity	n	AflB1		AflB2		AflG1		AflG2	
		c	a ( $\mu\text{g kg}^{-1}$ $\pm 0.70$ )	c	a ( $\mu\text{g kg}^{-1}$ $\pm 0.52$ )	c	a ( $\mu\text{g kg}^{-1}$ $\pm 0.75$ )	c	a ( $\mu\text{g kg}^{-1}$ $\pm 0.41$ )
Manufactured ration	8	2	110.89	1	10.23	5	225.83	3	31.53
Groundnut cake meal	26	13	103.63	12	36.76	7	51.89	8	33.49
Wheat bran	11	1	21.84	2	5.03	3	23.49	3	38.41
Crushes sorghum	11	2	206.70	1	5.46	2	136.91	0	-

n: Total number of samples; c: Number of contaminated samples; a: Concentration average of aflatoxin

The level of aflatoxins distribution in all types of the feeds investigated in the present study showed the following order:  $\text{AflB}_1 > \text{AflG}_1 > \text{AflB}_2 > \text{AflG}_2$ . This order is consistent with that obtained for peanut and peanut products in Sudan (Younis and Kamal, 2003).

Aflatoxin contamination has also shown seasonal variation. The highest contamination being in summer, where aflatoxin was detected in 78.95% of samples; followed by autumn (66.67%), while in winter the least contamination prevailed (47.37%). This is consistent with the view that the production of aflatoxin increased when climatic conditions such as high temperature and high relative humidity prevail (Murphy *et al.*, 2006).

**CONCLUSION**

The results obtained in the present investigation have shown that aflatoxin contamination in these feed samples is alarmingly high. This is evidently posing a dangerous problem to the poultry and livestock industry as well as to human health.

Lastly, a wide range of harmful effects and chronic toxicity in Sudanese animals can be easily overlooked and a serious economic loss could be expected with the increased exposure to disease problems due to immunosuppression. Further, it has been pointed out that aflatoxin contamination of feed of food-producing animals can result in residues of ingested aflatoxins or its metabolites in edible tissues such as meat, milk and egg (Charoenpornsook and Kavisarasai, 2006).

**RECOMMENDATION**

Furthermore, investigations are recommended to be carried out routinely to study contamination of food and feed commodities by all types of mycotoxins.

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