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ORIGINAL ARTICLE

Mycotoxins Contamination of Animal Feeds and Feed Ingredients Available in Haryana

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Abstract

The present study was undertaken to know the extent of mycotoxin contamination of animal feed and feed ingredients available in Haryana. The samples of animal feeds and feed ingredients (n=110) were collected and analysed for the presence of mycotoxins (aflatoxin B_1 and ochratoxin A). The highest level of aflatoxin B_1 detected was in maize (120 ppb), followed by groundnut cake (109 ppb), cottonseed cake (103 ppb), compounded feed (101 ppb), barley (100 ppb), mustard cake (95 ppb), sorghum (91 ppb), soybean meal (90 ppb), sunflower cake (88 ppb), wheat bran (62 ppb) and rice bran (55 ppb). The overall average content of aflatoxin B_1 in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 95.30, 67.90, 65.70, 25.80, 27.60, 88.10, 65.40, 41.90 and 51.20, 77.30 and 73.70 ppb, respectively. The overall average content of ochratoxin in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 42.30, 32.40, 27.20, 15.20, 22.40, 24.90, 15.20, 10.00, 15.30, 16.70 and 19.40 ppb, respectively. The ochratoxin level of contamination tended to be higher in cereals (maize, barley, sorghum) as compared to other feed ingredients and compounded animal feeds. The highest level of ochratoxin detected was in maize (82 ppb), followed by sorghum (74 ppb), barley (72 ppb), wheat bran (46 ppb), groundnut cake (46 ppb), cottonseed cake (45 ppb), compounded feed (45 ppb), rice bran (42 ppb), mustard cake (42 ppb), soybean meal (35 ppb) and sunflower cake (35 ppb). It was concluded that due to high level of mycotoxin contamination in animal feed and feed ingredients the situation is alarming as far as carry-over of mycotoxins from feed to milk is concerned. The ochratoxin level of contamination tended to be higher in cereals (maize, barley, sorghum) as compared to other feed ingredients and compounded animal feeds.

Keywords: Mycotoxin, Aflatoxin, Ochratoxin, Feed ingredient, Buffalo feed.

1. Introduction

Mycotoxins are often found as natural contaminants in animal feed and raw feed ingredients (Singh and Shrivastav, 2011a; Khan *et al*., 2011; Johri and Sadagopan, 1984; Johri *et al*., 1986). Aflatoxin and ochratoxin are natural contaminants of animal feeds and feedstuffs; and may cause large economic losses on animal production (Battacone *et al*., 2010). Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. There are four main types of naturally occurring aflatoxins i.e. B_1, B_2, G_1 and G_2. While AFB_1 is the most toxic in the group and the toxicity is in the order of $B_1>G_1>B_2>G_2$ (Dorner, 2004). The predominant signs of chronic aflatoxicosis in ruminants are feed refusal, reduced growth rate, decreased milk production and decreased feed efficiency. In addition, listlessness, weight loss, rough hair coat and mild diarrhea may occur. Anemia along with bruises and subcutaneous hemorrhage are symptoms of aflatoxicosis. The disease may also impair reproductive efficiency, including abnormal estrous cycles (too short or too long) and abortions. Other symptoms include impaired immune response, increased susceptibility to other diseases and rectal prolapse. Aflatoxin B_1 (AFB_1) present in feed of lactating animals gets transformed to 4-hydroxylated metabolite in liver and is excreted in milk as aflatoxin M_1(AFM_1). The presence of AFM_1 in milk poses a major risk for humans, especially children, as it can have immunosuppressive, mutagenic, teratogenic, and carcinogenic effects (Sefidgar *et al*., 2011). However, recent study show that aflatoxicol is also excreted with milk, aflatoxicol is the major metabolite of aflatoxin B_1 produced by microorganisms of the rumen flora, however AFM_1 is from hepatic origin. The carcinogenic potency of AFM_1 is almost as high as that of AFB_1, and the toxicological properties are generally comparable. On account of carcinogenicity of aflatoxin B_1, the only mycotoxin legislated in milk is its metabolite, AFM_1. Another important mycotoxin from feed contamination point of view is ochratoxin A, which is produced by several species of *Aspergillus* (*Aspergillus ochraceous*) and *Penicillium* (*Penicillium viridacatum*). Ochratoxin A is chemically defined as 7-carboxyl-S-chloro-8-hydroxyl 3, 4-dihydro-3-R-methyl isocoumarin linked to L-β-phenylalanine. The mode of action of OTA is not very clear but its structural similarity to phenylalanine and it's inhibition of several enzymes and processes dependent on phenylalanine suggest that OTA acts by disrupting phenylalanine metabolism. In ruminants, OTA is rapidly degraded in the rumen and thus thought to be of little importance besides young pre-ruminant calves where chronic exposure and acute toxicities are thought to occur in cattle. OTA in the rumen is converted into phenylalanine and ᾀ-OTA, the latter is less toxic but retaining some genotoxicity. Despite its metabolism in the rumen resulting in the formation of ochratoxin α, small amounts have been found in bovine milk (Breitholtz-Emanuelsson *et al.*, 1993). Reduced milk production, diarrhoea and kidney damage are some of the signs and symptoms of ochratoxin toxicity in dairy animals (Whitlow *et al.*, 2000). The objective of the present investigation was to study the extent of mycotoxins (aflatoxin and ochratoxin) contamination of animal feed and feed ingredients available in the state of Haryana.

2. Materials and Methods

The samples of commonly used feed ingredients (maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake and cottonseed cake), and compounded animal feeds available in the state of Haryana were collected. The samples were collected from grain markets, selling agents, farmers keeping buffalo and animal feed retailers. The samples were ground through 1 mm sieve using a willy mill and subjected to chemical analysis in duplicate for the presence of mycotoxins (aflatoxin and ochratoxin) as per the method of AOAC (1990). The aflatoxin and ochratoxin standards used in the present study were procured from M/s Sigma Co. (U.S.A.).

3. Results and Discussion

The samples of compounded feeds and feed ingredients were analysed in duplicate for the presence of aflatoxin B_1 and ochratoxin A content and their average values are given in Table 1 and 3, respectively.

3.1 Aflatoxin B_1

The results showed that the highest level of aflatoxin B_1 detected was in maize (120 ppb), followed by groundnut cake (109 ppb), cottonseed cake (103 ppb), compounded feed (101 ppb), Barley (100 ppb), mustard cake (95 ppb), sorghum (91 ppb), soybean meal (90 ppb), sunflower cake (88 ppb), wheat bran (62 ppb) and rice bran (55 ppb) (Table 2). In the present study, the level of aflatoxin B_1 contamination was lower as compared to those reported in earlier studies (Johri *et al*., 1986; Singh and Shrivastav, 2011a; Johri and Sadagopan, 1984). However, the extent of aflatoxin B_1 contamination in the present samples was slightly higher than that reported by Freed *et al*. (2014). The results further revealed that the percentage of aflatoxin contamination in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples was 100, 80, 80, 60, 60, 100, 80, 60, 70, 90 and 90, respectively (Table 2). The average contents of aflatoxin B_1 positive samples of maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 95.30, 84.87, 82.12, 43.00, 46.00, 88.10, 81.75, 69.83, 74.42, and 85.88 ppb, respectively. The overall average content of aflatoxin B_1 in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 95.30, 67.90, 65.70, 25.80, 27.60, 88.10, 65.40, 41.90, 51.20, 77.30 and 73.70 ppb, respectively. Similarly, Singh *et al*. (2010) reported that 90% of the maize samples were found positive for aflatoxin B_1. The contamination level ranged from 0.00 to 0.80 ppm with an average of 0.14 ppm of AFB_1. Also, Singh and Shrivastav (2011b) reported that 98% of the maize samples were found positive for AFB_1 and the values ranged from 0.00 to 0.40 ppm with an average of 0.13 ppm. The aflatoxin values reported in the present study were lower than those of Singh *et al*. (2010); Singh and Shrivastav (2011b). The present study further revealed that overall average content of aflatoxin B_1 in rice bran

Table 1: Aflatoxin content of animal feeds and feed ingredients

Feed	1	2	3	4	5	6	7	8	9	10
Maize	95	101	93	120	80	135	70	98	79	82
Barley	00	75	100	89	98	00	90	75	81	71
Sorghum	91	90	00	81	77	00	75	82	73	88
Rice bran	00	35	42	00	51	30	00	00	55	45
Wheat bran	45	38	40	00	00	35	56	62	00	00
Groundnut cake	98	106	85	76	109	104	78	75	86	64
Mustard cake	95	70	76	95	92	82	75	69	00	00
Soybean meal	00	76	85	90	00	00	61	55	52	00
Sunflower cake	88	75	70	85	00	76	00	62	00	65
Cottonseed cake	100	69	73	103	95	96	80	79	00	78
Compounded feed	90	00	86	76	98	61	64	76	85	101

Table 2: Aflatoxin contamination (percentage and average) of animal feeds and feed ingredients

Feed	Total No. of samples analysed	No. of positive samples	Percent positive	Total content (ppb)	Av. content of positive samples (ppb)	Overall average (ppb)	Highest level detected (ppb)	Range (ppb)
Maize	10	10	100	953	95.30	95.30	120	70-120
Barley	10	8	80	679	84.87	67.90	100	0-100
Sorghum	10	8	80	657	82.12	65.70	91	0-91
Rice bran	10	6	60	258	43.00	25.80	55	0-55
Wheat bran	10	6	60	276	46.00	27.60	62	0-62
Groundnut cake	10	10	100	881	88.10	88.10	109	64-109
Mustard cake	10	8	80	654	81.75	65.40	95	0-95
Soybean meal	10	6	60	419	69.83	41.90	90	0-90
Sunflower cake	10	7	70	512	74.42	51.20	88	0-88
Cottonseed cake	10	9	90	773	85.88	77.30	103	0-103
Compounded feed	10	9	90	737	81.88	73.70	101	0-101

and wheat bran was the lowest among all the feed ingredients. This could be due to the non-availability of readily utilizable sugars from bran since brans and meals of animal origin do not support the production of aflatoxin (Johri and Sadagopan, 1984). Similar results were also reported by earlier researchers (Johri *et al.*, 1986; Singh and Shrivastav, 2011a). In the present study, the overall level of aflatoxin B_1 contamination was lower than that reported by Biomin (2018) for Asian feeds. Qualitatively, few samples of the collected feeds and feed ingredients were also contaminated with additional B_2, G_1 and G_2. This result revealed that both *Aspergillus flavus* and *Aspergillus parasiticus* were involved in aflatoxin contamination of these feeds and feed ingredients since *Aspergillus flavus* is usually reported to produce only aflatoxin B_1 and B_2, while *Aspergillus parasiticus* may produce aflatoxin G_1 and G_2 in addition to aflatoxin B_1 and B_2 (Dorner *et al.*, 1984). Singh and Shrisvastav (2011a) also reported similar results during their surveillance of aflatoxin contamination in animal feed in and around Bareilly district of Uttar Pradesh. With regard to aflatoxin B_1 contamination of compounded feed, the average content of aflatoxin B_1 positive samples was 81.88 ppb, overall average content was 73.70 ppb and the range of contamination was 0-101 ppb. The permissible level of aflatoxin is 20 ppb in feed and 0.5 ppb in milk by FDA. WHO proposes a maximum level of 0.5 ppb in milk. European Commission limit is 10 times lower i.e. 0.05 ppb (IARC, 2002). France limit is 0.03 ppb. Therefore, feed containing 30 ppb aflatoxin can produce milk residues above the FDA action level of 0.5 ppb. Considering that about 1-3% ingested AFB1 is converted into AFM_1 (Ali *et al.*, 1999), in addition, a model calculation for a worst-case situation of aflatoxin carry-over into milk was performed for the major milk-producing animal species, including dairy cattle, sheep, goats, camels and buffaloes; and included carry-over rates of 2% (assumed average) and 6% (high yielding cows) (European Food Safety Authority, 2004). Keeping in view the level of aflatoxin B_1 contamination in feed and feed ingredients; and aflatoxin carry-over rate into milk, it can be inferred that the situation is alarming as far as the milk contamination due to aflatoxin transmission is concerned.

3.2 Ochratoxin A

The results showed that the percentage of ochratoxin contamination in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, -

Table 3: Ochratoxin content of animal feeds and feed ingredients

Feed	1	2	3	4	5	6	7	8	9	10
Maize	00	62	00	70	00	82	74	66	69	00
Barley	00	60	69	00	72	00	68	00	55	00
Sorghum	00	00	00	51	00	64	74	50	00	53
Rice bran	00	00	42	00	00	40	36	34	00	00
Wheat bran	35	00	32	00	00	00	35	45	46	31
Groundnut cake	42	30	40	00	00	25	30	36	00	46
Mustard cake	25	00	00	00	00	36	29	00	42	20
Soybean meal	00	00	24	22	00	00	19	00	35	00
Sunflower cake	00	26	35	31	00	00	26	00	35	00
Cottonseed cake	00	25	28	00	34	45	35	00	00	00
Compounded feed	45	00	26	00	34	00	26	28	35	00

Table 4: Ochratoxin contamination (percentage and average) of animal feeds and feed ingredients

Feed	Total No. of samples analysed	No. of positive samples	Percent positive	Total content (ppb)	Av. content of positive samples (ppb)	Overall average (ppb)	Highest level detected (ppb)	Range (ppb)
Maize	10	6	60	423	70.50	42.30	82	0-82
Barley	10	5	50	324	64.80	32.40	72	0-72
Sorghum	10	5	50	272	54.40	27.20	74	0-74
Rice bran	10	4	40	152	38.00	15.20	42	0-42
Wheat bran	10	4	40	224	56.00	22.40	46	0-46
Groundnut cake	10	7	70	249	35.57	24.90	46	0-46
Mustard cake	10	5	50	152	30.40	15.20	42	0-42
Soybean meal	10	4	40	100	25.00	10.00	35	0-35
Sunflower cake	10	5	50	153	30.60	15.30	35	0-35
Cottonseed cake	10	5	50	167	33.40	16.70	45	0-45
Compounded feed	10	6	60	194	32.33	19.40	45	0-45

soybean meal, sunflower cake, cottonseed cake and compounded feed samples was 60, 50, 50, 40, 40, 70, 50, 40, 50, 50 and 60, respectively (Table 4). Fazekas *et al*. (2002) reported lower frequencies of barley and maize contamination compared to the present study. The average contents of ochratoxin positive samples of maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 70.50, 64.80, 54.40, 38.00, 56.00, 35.57, 30.40, 25.00, 30.60, 33.40 and 32.33 ppb, respectively. The average level of ochratoxin contamination reported in the present study was lower than that of Rafai *et al*. (2000) wherein 350 ppb for soybean meal and 320 ppb for maize contamination of ochratoxin was reported. The overall average content of ochratoxin in maize, barley, sorghum, rice bran, wheat bran, groundnut cake, mustard cake, soybean meal, sunflower cake, cottonseed cake and compounded feed samples were 42.30, 32.40, 27.20, 15.20, 22.40, 24.90, 15.20, 10.00, 15.30, 16.70 and 19.40 ppb, respectively. The ochratoxin level of contamination tended to be higher in cereals (maize, barley, sorghum) as compared to other feed ingredients and compounded animal feeds. The highest level of ochratoxin detected was in maize (82 ppb), followed by sorghum (74 ppb), barley (72 ppb), wheat bran (46 ppb), groundnut cake (46 ppb), cottonseed cake (45 ppb), compounded feed (45 ppb), rice bran (42 ppb), mustard cake (42 ppb), soybean meal (35 ppb) and sunflower cake (35 ppb). In the present study, the overall level of ochratoxin contamination was lower than that reported by Biomin (2018) for Asian feeds. The study further revealed that ochratoxin level of contamination tended to be higher in cereals (maize, barley, sorghum) as compared to other feed ingredients and compounded animal feeds. This finding was in agreement with that of JECFA (2008) wherein the Joint FAO/WHO Expert Committee on Food Additives has also emphasized the relevance of ochratoxin in human food mainly due to consumption of contaminated foodstuffs such as cereal grains. Likewise, the risk of intake of ochratoxin is much lower in buffaloes than in pigs and poultry species, because buffalo feeding is mostly based on forages and only partially on cereals, which are the feeds with the highest risk of contamination. In several ruminant species, such as cattle, buffalo, sheep and goat, ochratoxin transfer in meat and milk due to ingestion of ochratoxin contaminated feed is not very frequent under field conditions, as ochratoxin is degraded by rumen microorganisms into less toxic metabolites which are mainly excreted in urine and feces (Marquardt and Frohlich, 1992).

4. Conclusion

It was concluded that due to high level of mycotoxin contamination in animal feed and feed ingredients the situation is alarming as far as carry-over of mycotoxins from feed to milk is concerned.

The ochratoxin level of contamination tended to be higher in cereals (maize, barley, sorghum) as compared to other feed ingredients and compounded animal feeds.Therefore, mycotoxin contamination of feed is a complex problem in buffalo production due to the economic losses of decreased production performance; adverse effects on animal health and welfare; and the mycotoxin transfer into milk and meat of intoxicated animals.

5. Recommendation

Monitoring of aflatoxin B_1 in animal feed and aflatoxin M_1 in buffalo milk, should be encouraged and data on the carry-over rate of aflatoxin into milk should be generated based on modern production system for high producing buffaloes.

References

Ali N, Hashim NH and Yoshizawa T (1999). Evaluation and application of a simple and rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philipines. *Food Additives and Contaminants*, 16: 273-280.

AOAC (1990). Official methods of analysis. Association of official analytical chemists, Washington DC, USA.

Battacone G, Nudda A and Pulina G (2010). Effects of ochratoxin A on livestock production. *Toxins*, 2: 1796-1824.

Biomin (2018). Mycotoxins in main commodities. *Biomin World Mycotoxin Survey*. pp. 8.

Breitholtz-Emanuelsson A, Olsen M, Oskarsson A, Palminger I andHult K (1993). Ochratoxin A in cow's milk and in human milk with corresponding human blood samples. *Journal of AOAC International*, 76: 842-846.

Dorner JW (2004). Biological control of aflatoxin contamination of crops. *Toxin Reviews*, 23(2): 425-450.

Dorner JW, Cole RJ and Diener UL (1984). The relationship of *Aspergillus flavus* and *Aspergillus parasiticus* with reference to production of aflatoxin and cyclopiazonic acid. *Mycopathologia*, 87: 13-15.

European Food Safety Authority (2004). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to aflatoxin B_1 as undesirable substance in animal feed. Request No. EFSA-Q-2003-035.EFSA. Parma, Italy.

Fareed G, Khan SH, Anjum MA and Ahmed N (2014). Determination of aflatoxin and ochratoxin in poultry feed ingredients and finished feed in humid semi-tropical environment. *Journal of Advance Veterinary and Animal Research*, 1(4): 201-207.

Fazekas B, Tar AK and Zomborszky-Kovacs M (2002). Ochratoxin A contamination of cereal grains and coffee in Hungary in the year 2001. *Acta Veterinaria Hungarica*, 50: 177-188.

IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 82: 171-300. (IARC: Lyon, France).

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2008). Safety evaluation of certain mycotoxins in food.WHO Food Additives Series 59. Avaliable online: http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf.

Johri TS and Sadagopan VR (1984). Surveillance of aflatoxin B_1 content of poultry feedstuffs in and around Bareilly district of Uttar Pradesh. *Indian Journal of Poultry Science*, 19: 55-56.

Johri TS, Agarwal R and Sadagopan VR (1986). Surveillance of aflatoxin B_1 content of poultry feedstuffs in and around Bareilly district of Uttar Pradesh. *Indian Journal of Poultry Science*, 21: 227-230.

Khan SH, Shamsul H, Rozina S and Muhammad AA (2011). Occurrence of aflatoxin B_1 in poultry feed and feed ingredients in Pakistan. *International Journal of Agro Veterinary and Medical Sciences*, 5: 30-42.

Marquardt RR and Frohlich AA (1992). A review of recent advances in understanding ochratoxicosis. *Journal of Animal Science*, 70: 3968-3988.

Rafai P, Bata A, Jakab L and Vanyi A (2000). Evaluation of mycotoxin-contaminated cereals for their use in animal feeds in Hungary. *Food Additives and Contaminants*, 17: 799-808.

Sefidgar S, Mirzae M, Assmar M and Naddaf S (2011). Aflatoxin M_1 in pasteurized milk in Babol city, Mazanda-ran Province, Iran. *Iranian Journal of Public Health*, 40: 115-118.

Singh R and Shrivastav AK (2011a). Surveillance of aflatoxin B_1 content in poultry feed ingredients in and around Bareilly district of Uttar Pradesh. *Indian Journal of Animal Nutrition*, 28(3): 320-324.

Singh R and Shrivastav AK (2011b). Occurrence of aflatoxins in maize feed in Bihar. *Indian Journal of Poultry Science*, 46(3): 341-345.

Singh R, Shrivastava HP and Shrivastav AK (2010). Mycotoxin contamination in maize as poultry feed. *Indian Journal of Poultry Science*, 45(1): 108-110.

Whitlow LW, Diaz DE, Hopkins BA, Hagler WM, Lyons TP and Jacques KA (Eds) (2000). Mycotoxins and milk safety: The potential to block transfer to milk, Proceedings of Alltech's 16th Annual Symposium. Nottingham, UK. (Nottingham University Press: 391-408. Available at http://en.engormix.com/MA-mycotoxins/articles/mycotoxins-milk-safety-potential-t199/p0.htm.