Indian J. Vet. Pathol., 35(2) : 238-240, 2011

Analysis of feed ingredients for Afla and T-2 mycotoxins by ELISA in rural areas of Uttar Pradesh

R.B. Rai*, Shafiqur Rahman, Himanshu Dixit, Sweta Rai, Balvir Singh, Harendra Kumar[1], T. Damodaran[2] and K. Dhama

Division of Pathology, [1]Division of Animal Reproduction,
Indian Veterinary Research Institute, Izatnagar-243122
[2]C.S.S.R.I.-R.R.S., Lucknow (U.P.)

Received: 20.01.11; Accepted: 25.07.11

ABSTRACT

Rai, R.B., Rahman, Shafiqur, Dixit, Himanshu, Rai, Sweta, Singh, Balvir, Kumar, Harendra, Damodaran, T. and Dhama, K. (2011). Analysis of feed ingredients for Afla and T-2 mycotoxins by ELISA in rural areas of Uttar Pradesh. Indian J. Vet. Pathol., 35(2) : 238-240.

A survey of commonly available feed ingredients in rural households of Barabanki and Raebareli districts of Uttar Pradesh was conducted for the presence of afla and T-2 mycotoxins during winter months by commercially available ELISA kits. Out of 88 samples, comprising of 74 feed ingredients and 14 commercial poultry feed, 9% and 24% ingredient samples were negative for afla and T-2 toxins, respectively. Among the positive samples, 20% showed higher level of aflatoxins (20 ppb or more), whereas 35% samples had more than 100 ppb concentration of T-2 toxins. Generally, the problem of aflatoxin has been recorded more in the rainy season and thereafter, because of high relative humidity and optimum temperature. The present study highlighting the occurrence of two important mycotoxins (Aflatoxin and T-2) in feed ingredients of rural areas and the potential threat of mycotoxins on animal and human health requires an upsurge in the mycotoxin surveillance and monitoring even in colder seasons which would help timely follow up of suitable prevention and control strategies for alleviating the economic losses and reducing public health significance.

Keywords: Aflatoxins, mycotoxins, T-2 toxins

Mycotoxins, secondary metabolites produced by moulds, have been incriminated to incite adverse effects on livestock and human health worldwide particularly in the tropical and subtropical countries. Studies on mycotoxins have gained immense importance in recent years, as they are widely spread in foods and feeds, or in the raw ingredients used in their formulation posing serious threat of contamination of agricultural commodities[1]. Most of these mycotoxins belong to three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium.* Although over 300 different mycotoxins have been identified so far, those of most concern based on their toxicity and occurrence are, aflatoxins, ochratoxin, zearaleone, vomitoxin, fuminisins and T-2 toxin, which cause significant health implications, mainly through food and feed contamination[2,3].

Aflatoxins are toxic metabolites produced by a variety of moulds such as *Aspergillus flavus* and *A. parasiticus*. They are hepatotoxic, immunosuppressive and carcinogenic and can be present in grains, nuts, cotton seeds and other commodities associated with human food or animal feeds[3,4,5,6]. T-2 toxin, a type- A trichothecene, is mainly produced by *Fusarium sporotrichoides*. This mycotoxin occurs in grains such as wheat, maize, oat, barley, rice, beans and soyabeans as well as in some cereal based products. T-2 toxin inhibits protein synthesis and affects the actively dividing cells such as those lining the gastrointestinal tract, skin, thyroid and erythroid cells[5,7].

The presence of mycotoxins in feeds and its subsequent ingestion may decrease feed intake, can be pathogenic leading to serious health problems, immunosuppression and affect animal production performances besides the possible presence of toxic residues in milk, eggs, meat, etc. which is of public health concern[3,8,9,10]. The most favourable season for growth of fungus (and mycotoxins) on feed ingredients is rainy season when optimum ambient temperature and humidity is available. The present survey was conducted during peak winter months (when minimum fungus growth is observed) in Barabanki and Raebareli districts for assessing the status of two important mycotoxins (afla and T-2 toxins) in the rural household feed ingredients used for livestock as well as human consumptions. Winter season was chosen to conduct survey of mycotoxin contamination in feed ingredients to know the occurrences in lean periods of colder weather and whether mycotoxin still exists when generally it should not be.

Eighty eight samples comprising of 74 samples of grains (including broken grains) of wheat, rice, black gram, maize, oil cakes (mustard and ground nut cakes), bran, paddy husk and 14 feed samples from poultry sheds were collected during the month of January, 2010. Samples (100-150 g each) were collected randomly, in clean and sealable polythene bags/pouches, from the

***Corresponding author:** email: drrbrai@yahoo.co.in

individual rural household of sixteen villages of the two districts Barabanki and Raebareli of Uttar Pradesh, representing the feed and feedstuffs being utilized and consumed for animal and human consumption.

Samples were grounded, thoroughly mixed and processed using 70% methanol (1:5 W/V) as solvent for extraction. The filtered samples were screened for afla and T-2 mycotoxins using commercially available ELISA kit (Total aflatoxin ELISA kit, Affini Tech Ltd., Bentonville, AR and "Agra Quant T-2 Toxin Assay, Romers Lab., Austria) according to the manufacturer's instructions.

The feed analysis results for the detection of mycotoxin (afla and T-2) contamination of various feed ingredients using a rapid immunochemical screening methods of commercial ELISA kit are presented in the Table. In spite of peak winter months, only 7 feed ingredient samples were found negative for aflatoxin, whereas T-2 toxin was absent in 18 samples. Even commercially available feed samples showed presence of these toxins in 10 and 6 samples, respectively. Out of 74 feed ingredient samples, 15 (20%) had aflatoxin level above the permissible limit (20 ppb). In case of T-2 mycotoxin, 26 (35%) samples of feed ingredients showed values higher than the permissible limit (100 ppb). However, in case of commercially available poultry feed the level, except one each for afla and T-2 mycotoxin, was below the permissible limit.

Human and livestock consume many of these ingredients, as tested in the present study, in their daily diet and, thus, creating a constant stress on their vital organs[3,9,10 11]. Though, the quantity of toxins consumed and duration is important in creating impact on organs function, but unawareness of rural masses about these toxins may create problem. Reduced feed intake, recurring anorectic conditions, poor feed efficiency, and reduced production is often mistaken for parasitic infestations a one. Mycotoxins ingestion can be pathogenic leading to se ious health problems, such as liver, kidney or nervous system damage, nutritional interference, appetite loss, diarrhea, growth depression, and increased morbidity along with immunosuppression and thus increasing susceptibility to many infectious agents[8,9]. These can also be carcinogenic, mutagenic and teratogenic. They cause huge economic losses to livestock and poultry, and are of public health concern . Being generally heat stable, mycotoxins pose profound challenges to food safety, and most important toxin of public health concern are aflatoxins. Aflatoxins, are the major mycotoxin that affect animals, and are highly immunosuppressive[3,6]. These generally affect chicks, poults, piglets, pregnant sows and calves. The T-2 toxin in dairy animals has been associated with feed refusal, production losses, gastro-enteritis, intestinal hemorrhages and death. In birds and calves it has been reported to impair the immune response causing decline in egg production, feed consumption and weight loss[12].

From the Indian subcontinent, several reports are available on occurrence of mycotoxin contamination particularly to aflatoxin and T-2 mycotoxin in variety of feed and food-stuffs viz. cereals, oil seed cakes (groundnut, sesame) vegetable(s) and fruits[6,13,14,15,16,17,18]. Analyses of 4818 samples of agricultural commodities, comprising cereals, compound feeds and other ingredients revealed high quantities aflatoxins in oilseed cake (groundnut) (96.35%), and 8620 ppb of aflatoxin B1 was observed in maize[15]. The ICMR multicentric study on the occurrence of aflatoxin contamination in risk commodities namely, maize and groundnut showed that 21 % of groundnut samples and 26 % of maize samples analysed exceeded Indian tolerance limits of 30 ¼/kg [16]. Public health implications associated with consumption of mould-damaged feed and food-stuffs has also been reported[19]. Mycotoxins analyses using ELISA and thin-layer chromatography (TLC) revealed mycotoxin contamination in 69 (34.8%) maize samples from Karnataka region[17]. In punjab, a 6.52% prevalence of tricothecenes in 46 suspected feed samples has been reported[20]. Recently, Sharma and Ahamad[6] reviewed the occurrence of aflatoxin contamination in various feed and food ingredients from several states of the country, with level ranging from 10-7750 ppb, with

Table. Concentration of Afla and T-2 mycotoxin in food and feed stuffs collected from different places of Barabanki and Raebareli districts

Samples	Aflatoxin (nos.)	T-2 toxin (nos.)
Negative samples		
1. Feed Ingredients	7	4
2. Balanced feed	18	8
Positive samples		
Feed Ingredients	**67**	**56**
0-10 ppb	29	–
10-20 ppb	16	–
20-50 ppb	9	6
50-100 ppb	4	24
100-500 ppb	2	18
More than 500 ppb	–	8
Feed samples	**10**	**6**
0-10 ppb	7	–
10-20 ppb	2	–
20-50 ppb	1	1
50-100 ppb	–	4
100-500 ppb	–	1
More than 500 ppb	–	–

contamination rates of upto 100%, mostly being around average of 30-40%. An accurate level of mycotoxin contamination in feed ingredients and other commodities/feedstuffs need to be strictly monitored by exploiting rapid and new generation tools.

In conclusion, the present report highlights the aflatoxin and T-2 mycotoxin contamination of feed ingredients during winter season being used for animal and human consumption in the rural areas of the two districts of Uttar Pradesh. Generally, the problem of mycotoxins has been recorded more in the rainy season and thereafter, because of high relative humidity and optimum temperature. However, in the present study mycotoxin contamination of feed ingredients was observed still beyond permissible levels in the winter season, which warrants that mycotoxins need to be monitored throughout the year. These two mycotoxins have considerable impact on animal health and production and known public health significance, therefore more surveys on their occurrences need to be emphasized covering different geographical regions of the country, along with strengthening the diagnostic facilities so as to timely adapt suitable prevention and control strategies for alleviating the health impacts on animals as well as humans. Strong measures need to be implemented in the rural areas particularly in these regions surveyed that could hinder the growth of toxigenic fungi in feed and other food materials like improving the storage methods, harvesting practices and handling of crops.

ACKNOWLEDGEMENTS

Authors are thankful to National Agricultural Innovation Project (NAIP) Project, ICAR, Delhi for financial support and Director, IVRI for providing necessary research facilities to carry out the present research work.

REFERENCES

1. Glenn AE. 2007. Mycotoxigenic *Fusarium* species in animal feed. In: *Fusarium* and their toxins: Mycology, occurrence, toxicity. control and economic impacts. Morgavi DP and Riley RT (Eds), *Anim. Feed Sci. Technol*., **137**: 213–240
2. Binder EM, Tan LM, Chin LJ, Handl J and Richard J. 2007. Worldwide occurrence of mycotoxin in commodities feeds and feed ingredients. *Anim. Feed Sci. Technol.,* **137:** 265-282.
3. Dhama K, Chauhan, RS, Mahendran M, Singh KP, Telang AG, Singhal L and Tomar S. 2007. Aflatoxins- Hazard to livestock and poultry production: a review. *J. Immunol. Immunopathol.,* **9**(1&2): 1-15.
4. IARC 1993. Monographs on the evaluation of carcinogenic risks to humans: Some naturally occurring substances, food item and constituents, heterocyclic aromatic amines and mycotoxins. *International Agency for Research on Cancer. World Health Organisation, Lyon,* **56:** 397-333.
5. JECFA 2001. T-2 and HT-2 toxins. Monographs and Evaluations No. 47, (displayed 23 November 2007). Available at http://www.inchem.org/documents/jecfa/jecmono/v47ie06.htm
6. Sharma AK and Basheer Ahmad D. 2008. Aflatoxicosis: an overview of present Indian scenario. National Workshop on current status, diagnosis and management of mycotoxicosis in livestock and poultry. *Souvenir*, pp: 1-16.
7. Desjardins AE. 2006. Fusarium mycotoxins: chemistry, genetics and biology. *American Phytopathological Society.* St. Paul, MN, USA. pp 260.
8. Gareis M and Wolff J. 2000. Relevance of mycotoxin contaminated feed for farm animals and carryover of mycotoxins to food of animal origin. *Mycoses*, **1**: 79-83.
9. Vogel DS and Jimenez VLC. 2006. Mycotoxins in public health. *Rev. Salud. Publica*., **1**: 129-135.
10. Jand SK, Kaur P and Sharma NS. 2005. Mycoses and mycotoxicosis in poultry: A review. *Indian J. Anim. Sci.,* **75**(4): 465-476.
11. Pandey VV, Kurkure NV and Bhandarkar AG. 2006. Effect of T-2 toxin on growth, performance and haematobiochemical alterations in broilers. *Indian J. Exp. Biol.*, **44:** 86-88.
12. Krnjaja V, Levic J and Stankovic S. 2009. Ubiquity of toxigenic fungi and mycotoxins in animal feeds in Republic of Serbia. *Biotech. Anim. Husbandry,* **25:** 477-491.
13. Bilgrami KS, Sahay SS, Srivastava AK and Rahman MF. 1990. Incidence of zearalenone, DON and T-2 toxin producing strains of Fusarium sp. on food items. *Proceedings of the Indian National Science Academy, Part B, Biol. Sci*., **56**(2): 223-227.
14. Reddy GL and Reddy SM. 1994. Incidence of tricothecene mycotoxin-producing fungi in oil seed cakes. *Indian J. Anim. Nutri.,* **49(**195): 213-217.
15. Dhavan AS and Choudhary MR. 1995. Incidence of aflatoxins in animal feed stuffs: a decade's scenario in India. *J. A.O.A.C. International*, **78**(3): 693-698.
16. Vasanthi S and Bhat RV. 1998. Mycotoxins in foods - occurrence, health & economic significance & food control measures. *Indian J. Med. Res*., **108**: 212-224.
17. Janardhana GR, Raveesha KA and Shetty HS. 1999. Mycotoxin contamination of maize grains grown in Karnataka (India). *Food Chem. Toxicol.,* **37**(8): 863-8.
18. Chandrasekharan D. 2005. Mycotoxin contamination in feed and their impact on livestock production and products. Compendium of National Seminar-2005, SRDDL, IAH&VB, Bangalore. pp. 17-24.
19. Bhat RV, Beedu SR, Ramakrishna Y and Munshi KL. 1989. Outbreak of tricothecene mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir valley, India. *Lancet*, **1**(8628)**:** 35-37.
20. Rana JS and Singh B. 1991. Prevalence of mycotoxicosis in poultry in Punjab. *Indian J. Anim. Sci*., **61**(7): 671-676.