IMPORTANCE OF INTERACTIONS AMONG MYCOTOXINS IN POULTRY FEED

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Introduction

Anti-nutritional factors depress nutrient quality of the feed and hence the health and the performance of poultry. Mycotoxins are one such anti-nutritional factor present in feed ingredients and complete feed. They are the secondary toxic metabolites produced by various genera of fungi. Fungi that produce mycotoxins of significance in poultry industry include *Aspergillus, Fusarium* and *Penicillium*. Though hundreds of mycotoxins have been implicated in animal disorders, the most significant in India include aflatoxins, ochratoxins, citrinin, T-2 toxin, deoxynivalenol and zearalenone (Table 1).

Traditionally, aflatoxin has been considered as a synonym to mycotoxin mainly due to: 1. Availability of extensive data on the occurrence of aflatoxin and its adverse effects on poultry, and 2. Lack of analytical procedures and therefore, awareness among poultry farmers on other mycotoxins. Today in India, however, the awareness on mycotoxins other than aflatoxin has improved and poultry farmers in some countries are considering analyzing their finished feed for mycotoxins such as ochratoxins, T-2 toxin, and zearalenone in addition to aflatoxins.

A question still looms around? Does the analysis of feed for the above mycotoxins is complete and the answer is no. It is not possible to analyze for all the mycotoxins present in feed as it is laborious and time consuming. As a result mycotoxin-contaminated material might have been fed to the poultry before the farmers receive the extensive analytical reports. To avoid the economic losses due to mycotoxins in poultry, it is advisable to assume that if one mycotoxin is detected in feed there are all possibility that many more exist. The presence of aflatoxins is a certain indication of the presence of cyclopiazonic acid as both can be produced by same *Aspergillus* species such as *Aspergillus oryzae*. Similarly presence of T-2 toxin is a certain indication of many trichothecene mycotoxins such as diacetoxyscirpenol, HT-2 toxin, etc. Another good example for this is the co-occurrence of aflatoxin and ochratoxins. In tropical countries ochratoxins are mainly produced by *Aspergillus* fungi which can also produce aflatoxins and cyclopiazonic acid (DeVries et al., 2002).

Table 1. ***Major fungi genera with associated mycotoxins***

Fungi Genera	Associated Mycotoxins
Aspergillus	Aflatoxins, ochratoxins, cyclopiazonic acid, patulin, sterigmatocystin, gliotoxin, citrinin
Penicillium	Ochratoxins, citrinin, patulin, penicillic acid, cyclopiazonic acid, penitrem A, griseofulvin, PR toxin
Fusarium	Fumonisins, moniliformin, zearalenone, zearalenol, deoxynivalenol, nivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, T-2 toxin, iso T-2 toxin, acetyl T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenon-X, diacetoxyscirpenol, sciepentriol, 15-acetoxyscirpentriol, neosolaniol, fusaric acid
Claviceps	Ergot alkaloids

Prevalence of Mycotoxins in India

The widespread prevalence of multiple mycotoxins today in India may be due to:

Improved analytical procedures which have increased the chances of mycotoxin detection in conventional ingredients and complete feed

Increased use of by-products and alternative feed ingredients in an effort to reduced feed costs, which are likely also to have a higher incidence of mycotoxins

Changes in global climatic conditions which are more conducive to the growth of mould and subsequent mycotoxin production.

Ingredients used in livestock diets are derived from crops grown in different climatic conditions

The global trade in feedstuffs which means ingredients used in livestock diets are derived from crops grown locally and imported and hence subjected to different conditions during growing, storage and transport (Swamy, 2003)

Several recent surveys of the prevalence of mycotoxins indicate the seriousness of the mycotoxin problem in the Asia-Pacific Region. During the years 2004-2005, a survey was conducted to study the incidence of aflatoxin, ochratoxin and T-2 toxin in different feed ingredients and finished feeds collected from different states of the country. Out of 984 samples analyzed, 824 samples were found to be positive for the presence of aflatoxin, ochratoxin and T-2 toxin (Devegowda et al., 2005) (Table 2). 91, 94, 97 and 97% of cereals, cereal byproducts, oilseed meals and finished feeds, respectively, were tested positive for mycotoxins. These authors reiterated that aflatoxins are not the only problem in the region but also ochratoxins and T-2 toxin.

Table 2. ***Co-occurrence of aflatoxin B_1, ochratoxin A and T-2 toxin in feed ingredients and finished poultry feeds in India (2004-2005)***

Ingredients / feed	Number of positive samples in respective range of mycotoxin concentrations					
	Aflatoxin B_1, ppb	Ochratoxin A, ppb			T-2 toxin, ppb	
	0-50	50-100	0-50	50-100	0-50	50-100
Cereals	88	4	68	44	72	44
Cereal byproducts	48	8	4	16	32	8
Oilseed meals	152	8	64	40	60	24
Finished feed	328	76	200	80	268	96

Over a 5 year period, Chandrasekaran et al. (2002) assayed 7,173 samples of oil cakes, 3,842 samples of complete feed and 2,463 cereals for the presence of Ochratoxin A, citrinin and aflatoxin. Ochratoxin was detected in all samples while aflatoxin was found in 90% of the samples - **Table 3.**

Table 3. ***Co-occurrence of ochratoxin A with aflatoxin B_1 in complete feed in India***

Ochratoxin A (ppb)

	Aflatoxin B_1 (ppb)		Total sample no.	
	Negative		1-20	21-50
1-20	4	69	6	79
21-50	1	25	1	27
51-100	2	18	6	26
101-200	4	3	4	11
>200	3	0	0	3
Total sample no.	14	115	17	146

These and other surveys highlight clearly that aflatoxin is not the only mycotoxin that should be considered a threat to animal health and performance but also mycotoxins such as DON, zearalenone, ochratoxins, citrinin, T-2 toxin and fumonisins. Since these mycotoxins rarely occurs single, any solution considered must be capable of addressing the broad range of mycotoxins that may be present.

Impact of increasing raw material prices on animal mycotoxicoses

Mycotoxin surveys from around the world indicate that protein sources such as rapeseed meal, cottonseed meal, ground nut cake, sunflower cake, copra meal and palm kernel meal are more susceptible to mycotoxin contamination as compared to conventional raw materials (eg: soybean meal). Owing to high prices of conventional raw materials during certain years, feed manufacturers have been forced to opt for alternatives to soybean meal and this has increased the potential mycotoxicoses for livestock species. Similarly the cost of maize has forced to look at other energy sources, including by-products such as rice bran, wheat bran and screenings. Many mycotoxins are concentrated in the outer covering of the seeds and therefore, increase the chances of mycotoxin related problems when such materials are used in animal rations. For example, during the milling process DON fractionate with the highest concentration in the bran and lowest in the flour (Lee et al., 1987). Mycotoxins from these by-products in combination with mycotoxins from more traditional ingredients can result in toxicological interactions.

Toxicological Interactions

Co-occurrence of mycotoxins is common in feed ingredients (and complete feed).

The toxicity responses and clinical signs observed in poultry when more than one mycotoxin is present in feed are complex and diverse. Symptoms typical of mycotoxicosis are often seen in poultry despite analysis of the feed indicating only very low concentrations of individual mycotoxins.

Mycotoxins in combination exert a greater negative impact on health and productivity of animals than their individual effects (Swamy, 2003).

Mycotoxin interactions can be additive, synergistic or antagonistic.

Interactions can alter clinical signs, resulting in a set of diagnostic characteristics that differ from the sum of individual effects.

This subsequently makes field diagnosis of mycotoxin interactions difficult and emphasizes the need to characterize mycotoxin interactions in detail.

Interactions also pose challenges to the development of uniform methodologies for remediation of mycotoxin contamination. Although a decontamination protocol may effectively reduce the detectable levels of one mycotoxin, another mycotoxin may persist at harmful concentrations.

Some of the important toxicological interactions observed among various mycotoxins are given in **Table 4.**

It is important, therefore, that a mycotoxin adsorbent should be capable of adsorbing several mycotoxins simultaneously.

Table 4. ***Toxicological interactions among mycotoxins***

Mycotoxins	Poultry Species	Type of interaction	References
Aflatoxin & ochratoxin A	Broiler chicks	Synergistic	Huff and Doerr (1981)
Raju and Devegowda (2000)			
Aflatoxin & DAS	Broiler chicks	Synergistic	Kubena et al.,
(1993)			
Aflatoxin and DON	Broiler chicks	Additive	Huff et al., (1986)
Aflatoxin and T-2 toxin	Broiler chicks	Synergistic	Huff et al., (1988)
Ochratoxin A and T-2 toxin	Broiler chicks	Additive/synergistic/ Antagonistic	Kubena et al., (1989a)
Raju and Devegowda (2000)			
Ochratoxin A and citrinin	Broiler chicks	Antagonism	Manning et al.,
(1985)			
DON and moniliformin	Turkey poults	Moniliformin effect only	Morris et al., (1999)
DON and T-2 toxin	Broiler chicks	Synergistic	Kubena et al.,
(1989b)			
DON and ochratoxin A	Broiler chicks	< than additive / antagonistic	Kubena et al., (1988)
Fumonisin B_1 and moniliformin	Broiler chicks	Additive	Javed et al., (1993)
Fumonisin B_1 and T-2 toxin	Turkey poults	Additive	Kubena et al., (1995)
Fumonisin B_1 and T-2 toxin or DON	Broiler chicks	Additive	Kubena et al., 1997

Mycotoxin Adsorbents

Various approaches have been identified to reduce or prevent the adverse effects of mycotoxins on animal health and production. The most practical method is the inclusion of mycotoxin adsorbents in feed. There has been a misconception that mycotoxin adsorbents adsorb mycotoxins in feed. This is not true. When the feed is ingested and subsequently digested in GIT, mycotoxins are released from the feed matrix. The released mycotoxins are absorbed across the intestinal epithelium, enter the blood stream and transported to various organs causing cellular malfunction. When an effective mycotoxin adsorbent in used in the feed, it adsorbs the released mycotoxins in the GIT and they are safely excreted in feces, thereby preventing their transport to target organs. The net effect is a reduction in the dose of absorbable toxin to a concentration that does not adversely affect animal performance (Swamy, 2004). Most extensively studied mycotoxin binders include clay binders or carbon-based organic polymers - **Table 5.**

Table 5. ***Clay binders (HSCAS, bentonites, zeolites):***

Mycotoxins	No. of trials	References
Aflatoxins	9	Aravind et al., 2004; Girish and Devegowda, 2004; Khajarem and Khajarem, 1999; Raju and Devegowda, 2000; Reddy and Devegowda, 2004; Stanley et al., 1993, 2004; Valarezo et al., 1998; Swamy and Devegowda, 1998
Aflatoxin + ochratoxin + T-2 toxin	1	Raju and Devegowda, 2000
Aflatoxin + ochratoxin + T-2 toxin + zearalenone	2	Aravind et al., 2003; Santin et al., 2004
T-2 toxin	3	Dvorska, 2004; Girish and Devegowda, 2004; Kruk et al., 2004;
Aflatoxin + ochratoxin + T-2 toxin + DON + fumonisins	1	Vieira et al., 2004.
Fumonisin B_1 + T-2 toxin	1	Papazyan et al., 2004
Diacetoxyscirpenol	1	Pavicic et al., 2001
Ochratoxin A	1	Devegowda and Aravind, 2004
Fusarium mycotoxins	5	Swamy et al., 2002; Swamy et al., 2004a,c; Chowdhury and Smith, 2004

Although mineral binders are relatively low priced, they offer very limited protection against mycotoxins for several reasons (van Kessel and Hiang-Chek, 2001):

1. Some clay binders are processed and some are crude. The efficacy of such products is variable.

2. Mineral mycotoxin binders, such as aluminosilicates, are capable of binding only one specific mycotoxin (most commonly aflatoxin). Such products are not, therefore, effective against mycotoxins of varying molecular weight and polarity. It has been scientifically proven that clay binders are not effective against T-2 toxin, ochratoxins, DON, cyclopiazonic acid, zearalenone, diacetoxyscirpenol, fumonisins and ergotamine (DeVries et al., 2002).

3. Clay binders offer low specificity and so must be used at high level of inclusion to be effective.

4. The particles of clay binders expand as they come in contact with water. Because of this expansion, small molecules, including aflatoxins, are absorbed into the particle. This mode of action, however, has two important disadvantages.

   a. Clay binders can bind nutrients, such as minerals and vitamins, as well as aflatoxins (Chestnut et al., 1992; Kramer et al., 1993).

   b. Aflatoxin is absorbed, like a sponge, but not actually bound to the mineral particle. In practice, this means that after some time toxins can return to the GIT.

5. Clay products are not capable of adsorbing mycotoxins at different pH of GIT. One of the binding mechanisms of aflatoxins to HSCAS is by hydrogen bonding at acidic pH but release the toxin when pH becomes neutral or alkaline.

6. Very limited numbers of *in vivo* studies are available on clay binders and most of these studies have employed pure mycotoxin, which does not reflect the actual field mycotoxin problem.

Organic polymers: Recent progress in yeast biotechnology and carbohydrate chemistry has opened new avenues for tackling mycotoxin problems. In 1993, researchers supplemented an aflatoxin-contaminated broiler diet with 0.05 to 0.2% live yeast (Yea-Sacc[1026]) and reported significant improvement in the weight gain and feed efficiency (Stanley et al., 1993). Subsequent research demonstrated that the inner cell wall fraction of yeast was responsible for adsorbing mycotoxins and preventing mycotoxicoses (Raju and Devegowda, 2000).

Salient Characteristics of Esterified Glucomannan as an Ideal Mycotoxin Adsorbent

1. Ability to adsorb a wide range of mycotoxins
2. A Low Effective Inclusion Rate
3. Stability over a wide pH range
4. High capacity to adsorb high concentrations of mycotoxins
5. High affinity to adsorb low concentrations of mycotoxins
6. Ability to adsorb mycotoxins rapidly in GIT
7. Proven *in vivo* data: Ultimately, proving the worth of mycotoxin adsorbents must be through *in vivo* studies which are more representative than *in vitro* as it is very difficult to simulate the GIT conditions *in vitro* (van Kessel and Hiang-Chek, 2001), and conclusions are inaccurate. Unlike all other binders, Esterified Glucomannan has proven its efficacy in a large number of *in vivo* trials conducted in many livestock and poultry species around the world **(Table 5)**. Some of the recent *in vivo* studies are discussed in detail in the following section.

Conclusions

Several extensive surveys of mycotoxins in feed ingredients and complete feed in India have clearly identified the presence of ochratoxins, T-2 toxin, zearalenone, and citrinin in addition to aflatoxins.

The rising cost of the traditional mainstay ingredients, maize and soybean meal, has forced producers to consider greater use of by-products. This is likely to further increase the prevalence of mycotoxicoses since these alternative feed ingredients are more likely to contain mycotoxins. Trade in raw ingredients around the region exacerbates the problem and makes prediction of mycotoxin incidence difficult.

Livestock producers in the Asia-Pacific Region can minimize the economic losses caused by mycotoxins by using an effective adsorbent.

References: can be obtained from author upon request.