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Short Communication

In vitro binding ability of mycotoxin binder in commercial broiler feed

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In the present work an *in vitro* model was tested in evaluating an adsorbent to ameliorate the toxic effects of mycotoxins by quantifying free mycotoxins using thin layer chromatography analysis method. *In vitro* binding ability of a commercial binder (0.2%) on aflatoxin B_1 (AF) (500 ppb), ochratoxin A (OA) (1 ppm) and T-2 toxin (T-2) (2 ppm), when present alone or in combination, was evaluated at pH 4.5 and 6.5 in the diets. Binder showed significantly ($p < 0.05$) higher binding ability for AF (94.71%), whereas those recorded for T-2 (74.28%) and OA (63.13%) were moderate. Binding of each toxin decreased as the number of toxins in feed increased. Among 4.5 and 6.5 pH, higher binding ability for this binder was significantly ($p < 0.05$) noticed at 6.5 pH of the medium.

Key words: Aflatoxin, ochratoxin A, T-2 toxin, binder, *in vitro*.

INTRODUCTION

Animal feed ingredients and compounded feeds, by virtue of their high vital nutrients and moisture contents generously support the multiplication of moulds at all stages in the food chain, that is, production, harvesting, handling, processing and storage. Many of these moulds produce toxic metabolites some of which are known as mycotoxins. Aflatoxin (AF), Ochratoxin A (OA) and T-2 toxin (T-2) are secondary metabolites of *Aspergillus flavus*/-*Aspergillus parasiticus, Aspergillus ochraceus* and *Fusarium sporotrichoides,* respectively, are commonly encountered in animal feedstuffs. These mycotoxins when consumed in combination may show greater negative effects on the well being and productivity of broiler chickens than when consumed alone (Raju and Devegowda, 2002; Denli and Okan, 2006; Yegani et al., 2006). Many approaches have been attempted to counteract mycotoxicosis in poultry operations using single or combined physical, chemical, nutritional and biological methods. Though some of them have proved effective on some mycotoxins, the search is still on for a natural, cost effective and field applicable solution to this problem in poultry.

Herbal components like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and green algae (*Spirulina plantesis*) have been shown to counteract Mycotoxin and they also act as good antioxidants. Moreover, because of their non-toxicity, the use of these agents in preventing the effects of aflatoxin in chicken has been studied (Fanelli et al., 1985). The present trial was conducted to study the *in vitro* binding ability of Niltox (Zeus Biotech Limited, Mysore, India), a toxin binder which is claimed to degrade peroxides, amides and lacto rings of non-polar toxins and prevent DNA adduct formation and cellular damages by preventing oxidation of toxins (Soni et al., 1992).

MATERIALS AND METHODS

Mycotoxin binding efficacy of Herbal Binder (HB) was evaluated in toxin-contaminated feed under simulated *in situ* gastrointestinal (GI) tract environment of chicken. The composition of binder is as follows:

i.) Minerals (extra purified clay containing diatomaceous earth mineral) (15%).
ii.) Antioxidants (Curcuminoids extracted from Turmeric) (10%).
iii.) Enzymes (Epoxidase and Esterase) (75%).

Experimental design

Aflatoxin B_1 (500 ppb), OA (1 ppm) and T-2 (2 ppm) were studied individually and in combination with or without binder (0.2%) (Table

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1). In each of these treatments, the toxin adsorption activity of binder as in the fore and mid portions of GI tract of chicken in triplicates was tested at pH levels of 4.5 and 6.5.

Production and quantification of mycotoxins

AF, OA and T-2 were produced employing solid substrate fermentation as per the methods of Shotwell et al. (1969), Trenk et al., (1975) and Burmeister et al., (1971), respectively. The respective fungal cultures used were *A. parasiticus* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India), *A. ochraceus* NRRL 3174 (Source: National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 64604, USA) and *F. sporotrichoides* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India).

Mycotoxin content of the culture material was determined by thin layer chromatography as per AOAC (1995) in case of AF and OA and Rukmini and Bhat (1978) and Romer et al., (1978) in case of T-2.

Experimental procedure

Compounded broiler finisher (2994 Kcal/kg ME and 18.58% CP) feed weighing 25 g, was taken in a 250 ml Erlenmeyer flask and the required quantity of culture material was added to arrive at the desired level of toxin. Binder was added at a rate of 0.2% to these flasks whereas the feed in control flasks of the respective treatment was left untreated. Citric acid-sodium phosphate buffer (100 ml) of the desired pH (4.5/6.5) was added to each flask and the contents were incubated at 37°C for 3 h. From each flask, the content was filtered and dried at 37°C for 2 h, while the respective toxin was extracted from the content, further quantified and expressed the recovery rate in percentage.

The percentage difference in the toxin content between the beginning and at the end of trials in binder treated and control flasks were calculated. The binding of each toxin in different treatments was determined by subtracting the percentage difference in toxin content of the control flasks from that of the treated flasks in the respective treatments.

$$\text{Per cent toxin adsorption} = \left[\frac{B_T - E_T}{B_T} X\ 100\right] - \left[\frac{B_C - E_C}{B_C} X\ 100\right]$$

Where B_T = toxin content in the treated flasks at the beginning.
E_T = toxin content in the treated flask at the end.
B_C = toxin content in the control flasks at the beginning.
E_C = toxin content in the control flask at the end.

Statistical analysis

The data were subjected to ANOVA using General Linear Model procedure SAS Institute (2000), under factorial design for the main effects and interactions and randomized block design for the treatment effects. Data collected in percentages were converted into arc sine angles prior to statistical analysis. Means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSIONS

The percentage binding of AF, OA and T-2 by binder, either singly or in combination are presented in Table 1. Significant ($p < 0.05$) differences were noted in binding of different mycotoxins among the different dietary treatments. In diets containing the individual toxins, a signifycantly higher binding of AF was recorded when compared with that of T-2 and OA.

At pH 4.5, the highest percentage binding of mycotoxins was noticed for AF (90.68%), whereas the lowest binding ability was recorded for OA (61.73%). Results also indicate that at the pH of 4.5 in the combined treatments, the highest binding ability was noticed for AF+OA (62.30%) when compared with AF+OA+T-2 (5.33%).

At pH 6.5, highest binding percentage of mycotoxins was recorded for AF (94.71%); while on the contrary, treatment with OA provided the lowest binding percenttage of 63.13%. In diets containing the combined treatments with pH of 6.5, the binding ability for AF+OA (65.80%) was much higher when compared to that of AF+OA+T-2 (6.26%).

The diatomaceous earth is a powerful natural adsorbent and it might adsorb the toxins effectively through their polar ends of toxin (Gowda et al., 2008).

Curcumin induces drug metabolizing enzymes like glutathione-s-transferase and induction of enzymes results in efficient detoxification of cytotoxic or carcinogenic compounds (Shalini and Srinivas, 1987; Soni et al., 1992). While vitamin E and C are the main antioxidants that inhibit free radical damages in biological systems, curcumin removes these free radicals by producing a stable radical and thus its molecules that act as shuttle of scavengers for these radicals (Fanelli et al., 1985). This effect may be influenced in part by the nature of functional atomic groups present on the mycotoxin molecule.

The other commonly used binding agents such as aluminosilicates, activated charcoal, bentonite, mannnan-oligosaccharide etc. have been found to have varied effects on aflatoxin binding (Gowda et al., 2008), ochratoxicosis (Irina et al., 2007), fusarium toxicity (Yegani et al., 2006) and T-2 toxicosis (Raju and Devegowda, 2002). However, the ability of the toxin binder to bind mycotoxins depends on other factors such as pH, molecular arrangement and its geographic region of origin (Vieira, 2003).

Thus it could be construed that the binder used in this study has a broad-spectrum efficacy against the tested mycotoxins, mainly aflatoxin and will selectively inactivate certain mycotoxin molecules effectively especially at pH of 6.5 because the binder contains diatomaceous earth mineral, curcuminoids and enzymes. However, this binding ability towards each toxin decreased as the number of toxins increased.

This reduction in per cent binding might have been due to the interaction among the mycotoxins (Raju and Devegowda, 2002).

Table 1. Per cent mycotoxin binding *in vitro* by a commercial binder at two pH levels in different dietary treatments.

Treatments	AF			OA			T-2		
Mycotoxins	pH			pH			pH		
	4.5	6.5	$\bar{X}$	4.5	6.5	$\bar{X}$	4.5	6.5	$\bar{X}$
AF	90.68	94.71	92.69[a]	-	-	-	-	-	-
OA	-	-	-	61.73	63.13	62.43[e]	-	-	-
T-2	-	-	-	-	-	-	71.33	74.28	72.80[e]
AF+OA	62.30	65.80	64.05[b]	33.70	34.00	33.85[g]	-	-	-
AF+T-2	43.24	44.67	43.95[c]	-	-	-	46.14	46.39	46.26[f]
OA+T-2	-	-	-	34.08	34.05	34.06[g]	45.27	47.48	46.37[f]
AF+OA+T-2	33.47	32.43	32.95[d]	5.33	6.26	5.79[h]	9.10	9.70	9.40[h]
Average for each mycotoxin			58.41[x]			34.03[y]			43.70[z]
SEM			3.54			1.94			2.06

[a-h]Means of different mycotoxins in each treatment, bearing common superscript, do not differ significantly ($p < 0.05$).
[x-z]Pooled means of each mycotoxin among the various treatments, bearing different superscripts differ significantly ($p < 0.05$).
AF: Aflatoxin B_1 0.5 ppm, OA: Ochratoxin A 1 ppm, T-2: T-2 toxin 2 ppm, SEM: Standard Error for the Mean.

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