Ability of Modified Glucomannan to Sequestrate T-2 Toxin in the Gastrointestinal Tract of Chicken*

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ABSTRACT : The ability of Modified Glucomannan (MG) to bind T-2 toxin (T-2) in the gastrointestinal tract has been tested *in vivo* by feeding 120 five-wk-old broiler chicken with the following six treatment diets, 1) Control diet; 2) Control+MG (0.1%); 3) Control+T-2 (500 ppb); 4) Control+T-2 (500 ppb)+MG (0.1%); 5) Control+T-2 (1,000 ppb) and 6) Control+T-2 (1,000 ppb)+MG (0.1%). Twenty birds were assigned to each treatment group, which had five experimental groups. Four birds of each experimental group were sacrificed at an interval of 30 min i.e. at 0, 30, 60, 90 and 120 min after feeding experimental diets. The whole gut contents of each bird were collected, dried and toxin concentration was determined. Percent T-2 recovered from the gut was significantly lower ($p<0.05$) in the groups fed MG at all the time intervals. The percent T-2 adsorbed by the MG at different T-2 levels (500 and 1,000 ppb) was 15.97 and 14.77, 22.53 and 22.67, 26.88 and 28.03, and 31.50 and 31.83 at 30, 60, 90 and 120 min, respectively. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 2 : 259-262)***

Key Words : T-2 Toxin, Absorbed Toxin, Adsorbed Toxin, Recovered Toxin and Modified Glucomannan

INTRODUCTION

The toxicokinetic studies in chicken indicate that the rate of absorption of T-2 toxin (T-2) from the gastrointestinal tract (GIT) is the highest among the trichothecenes (Chi et al., 1978; Prelusky et al., 1986). The T-2 concentration in circulation reaches maximum levels just over one hour after oral dosing (Feinberg and Mc Laughlin, 1989). Furthermore, previous studies indicate that within min after absorption, T-2 is distributed all over the body with the liver and kidney having the largest amounts (Ueno et al., 1971; Chi et al., 1978). In chickens, nearly 80% of T-2 is excreted from the body within 48 hours after dosing (Yoshisawa et al., 1980; Giroir et al., 1991). Aforementioned toxicokinetic findings give an idea about the possible stress chickens may undergo in a short course of time to eliminate the T-2 toxin from body, which may account for the acute toxicity of T-2 toxin in broilers and low LD_{50} (Chi et al., 1978; Hoerr et al., 1981).

The Modified Glucomannan (MG) derived from yeast cell wall has the ability to bind several mycotoxins including T-2 toxin (Raju and Devegowda, 2000; Arvind et al., 2003) and prevent the adverse effects in chicken (Devegowda et al., 1998; Manoj and Devegowda, 2000; Dvorska and Surai, 2001). An *in vitro* binding study conducted recently has shown MG to bind T-2 up to 33% (Dawson et al., 2001) and similar findings were found in our laboratory (Raju and Devegowda, 2002).

The crucial aspect of absorption of T-2 from the chicken GIT is the absorption of large amount of T-2 in between the crop and jejunum (Lun et al., 1988). This amounts to the fact that maximum absorption of T-2 takes place during the period between 15 to 90 min after consumption of contaminated feed. Therefore, a toxin adsorbent should be able to bind maximum quantity of toxin before feed reaches the duodenum, within 90 min after feeding, to effectively counteract T-2 toxicosis in chicken. The present experiment was conducted to test the T-2 binding ability of MG (Source: Alltech Inc., Kentucky 40536, USA) in gut conditions of chicken, and also to develop a simple and effective *in vivo* method to test the binding efficacy of a mycotoxin adsorbent.

MATERIALS AND METHODS

Production and quantification of T-2 toxin

The T-2 was produced employing solid substrate fermentation as per the procedure of Burmeister (1971). *Fusarium sporotrichoides* MTCC 1894 (Source: Institute of Microbial Technology, Chandigarh 160 036, India) was utilized to produce T-2.

The T-2 was extracted as per the method of Romer et al. (1978) and was quantified by TLC as suggested by Rukmini and Bhat (1978).

Experimental birds

One hundred and twenty five-wk-old broilers weighing around 1,200 g were randomly divided into six groups of 20 birds each. Each treatment group was allotted to one of the six dietary treatments at random. The birds in each group were housed individually in cages (30×45×45 cm) and were fed control diet for the first seven d. Feed was withdrawn for 12 h before feeding the experimental diets on the eighth d.

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Table 1. Composition of control diet

Ingredients (%)	
Yellow maize	65
Soybean meal	31.8
Mineral mixture[1]	3.2
Salt (g)	300
$A B_2 D_3 K$[2] (g)	15
B-complex[3](g)	20
Monocox[4](g)	50
DL-methionine (g)	100
Choline chloride[5](g)	100
Calculated composition	
Protein (%)	20.03
ME (kcal/kg)	2,908
Crude fiber (%)	3.86
Calcium (%)	1.13
Available P (%)	0.40
Lysine (%)	1.08
Methionine (%)	0.42

[1] Provides per kg: Ca; 90 g, P; 90 g, Mn; 440 mg, Zn; 330 mg, I; 150 ppm, Fe; 2,000 ppm, Cu; 250 ppm and Se; 45 ppm.
[2] Provides per g: A; 82,500 IU, D_3; 12,000 IU, B_2; 50 mg and K; 10 mg.
[3] Provides per g: B; 4 mg, B_6; 8 mg, B_{12}; 40 µg, E; 20 mg, Niacin; 60 mg and Calcium pantothenate; 12.5 mg.
[4] Provides per g: Maduramycin; 10 mg.
[5] Provides per g: Choline chloride; 600 mg.

Experimental design

Six dietary treatments were prepared in a factorial arrangement with three dietary levels of T-2 (0, 500 and 1,000 ppb) and two levels of MG (0 and 0.1%). Required quantities of the culture material and MG were added to the control diet to prepare the six experimental diets.

Experimental procedure and data collection

On the day of experiment, each experimental diet was fed to one of the six groups of chicks. The quantity of feed consumed by each bird was recorded to calculate the total quantity of T-2 consumed. Four birds from each treatment group were sacrificed by cervical dislocation immediately after feeding the test diets and at an interval of 30 min then onwards, i.e. at 30, 60, 90 and 120 min. By making two cuts on either side of keel bone, the abdominal cavity was exposed. The GIT running from esophagus to ceca was removed from the abdominal cavity. The gut contents were collected in a beaker by squeezing, and the GIT was cut open and contents were scraped into the respective beakers. Then, the gut was washed with distilled water and the washings were collected in the same beakers. The samples were dried in hot air oven at 50°C till the samples were dried and weight of the samples was recorded. The T-2 concentration in the dried samples was determined by TLC method and percent T-2 recovered from each sample was calculated.

Percentage of T-2 toxin adsorbed

The percent T-2 adsorbed by the MG was considered almost equal to the difference between the recovered toxin from the GIT of birds fed T-2 alone and the recovered toxin from the GIT of birds fed T-2 and MG.

The distribution of total T-2 consumed in birds fed T-2 alone was calculated as follows: - a fraction of T-2 consumed will be absorbed (ABt_1) into the gastrointestinal system and the remaining toxin is recovered (Rt_1) from the gut. Where as in case of birds fed T-2 and MG a fraction of T-2 consumed will be absorbed (ABt_2) into the gastrointestinal system, a part of T-2 is adsorbed (ADt) to MG and the remaining toxin is recovered (Rt_2) from the gut. Therefore,

a) In birds fed T-2 alone.

$$\text{Total T-2} = ABt_1 + Rt_1 \quad (1)$$

b) In birds fed T-2 + MG

$$\text{Total T-2} = ABt_2 + ADt + Rt_2 \quad (2)$$

From (1),

$$ABt_1 = \text{Total T-2} - Rt_1 \quad (3)$$

To calculate the ADt, it was presumed that ABt_2 was approximately equal to ABt_1 as the birds used in both the groups were of same age and similar weight. Furthermore, a study in our laboratory has shown that MG and T-2 complex is stable and bound toxin cannot be estimated by TLC (Unpublished data).

Consequently, From (2) and (3),

$$ADt = \text{Total T-2} - (ABt_1 + Rt_2) \quad (4)$$

From (1) and (4),

$$ADt = Rt_1 - Rt_2$$

Statistical analysis

The data collected were subjected to two-way ANOVA using the Graph Pad Prism computer program (1995). In the analysis of recovered toxin, individual bird was taken as an experimental unit and Turkey's mean comparison was used to test for statistical differences for recovered T-2. All the values expressed as significant were tested at $p<0.05$ level.

RESULTS AND DISCUSSION

The results on the percent T-2 recovered from the gut at different time intervals are presented in Table 2. The T-2 toxin was not detected in the group fed either control diet or control diet+MG (not presented in the table), this may be due to the fact that feed ingredients were screened for T-2 prior to compounding the feed and analyzed values did not show T-2 at detectable levels in both the diets. Similarly, T-2 toxin was not detected in gut contents at zero min, which

Table 2. T-2 toxin recovered from gut contents of broiler chicken fed different dietary treatments[1] (%)

T-2 toxin (ppb)	Modified glucomannan (%)	Feed sample[2]	30 min	60 min	90 min	120 min
500	-	98.20^{ax}	85.27^{bx}	69.85^{cx}	47.25^{dx}	35.65^{ex}
500	0.1	95.81^{ax}	69.3^{by}	47.32^{cy}	20.37^{dy}	4.15^{ey}
1,000	-	98.45^{ax}	83.17^{bx}	71.27^{cx}	50.23^{dx}	38.05^{ex}
1,000	0.1	96.22^{ax}	68.4^{by}	48.6^{cy}	22.2^{dy}	6.22^{ey}

[1] Values represent the mean of gut contents from four birds (n=4).

[2] Values represent the mean of four feed samples. [a-e] Means within a row having no common superscript differ significantly ($p<0.05$).

[x-y] Means within a column having no common superscript differ significantly ($p<0.05$).

Table 3. T-2 toxin adsorbed to Modified Glucomannan in the groups fed T-2 and Modified glucomannan[1] (%)

T-2 Toxin (ppb)	Modified glucomannan (%)	30 min	60 min	90 min	120 min
500	0.1	15.97	22.53	26.88	31.50
1,000	0.1	14.77	22.67	28.03	31.83

[1] Values represent the mean of four birds (n=4).

is not presented in the table. Thus indicating no residual T-2 toxin in the gut contents due to prior feeding. It has been reported that T-2 and its metabolites are completely excreted without any residues in the body (Chi et al., 1978). In the group fed T-2 (500 and 1,000 ppb) alone, differences were noticed in the recovered toxin at different time intervals, and results indicate progressive absorption of T-2 into the gastrointestinal system. The progressive reduction in recovery of T-2 may be attributed to its absorption and biotransformation. It is evident that T-2 is readily absorbed from the GIT, and absorption continues up to 120 min after consumption. These findings are supported by toxicokinetic studies of Feinberg and Mc Laughlin (1989) and Chi et al. (1978). The highest amount of absorption of T-2 was noticed during 60 to 90 min interval. This is presumed to occur due to the absorption of the toxin by enterocytes in small intestine and its conversion to other metabolites (Lun et al., 1988). Differences were also noticed in the percent T-2 recovered at different time intervals in the birds fed T2 and MG. This progressive reduction could be due to absorption of T-2 into the gastrointestinal system and/or adsorption of T-2 by the MG.

The percent recovery of T-2 in birds fed T-2 and MG was lower than the birds fed T-2 alone at all time intervals. Progressive increase in difference in T-2 recovery between these two groups was evident. This may be due to selective binding of T-2 by the MG and also indicates that the MG was able to bind T-2 right through the GIT.

The results on the percent adsorbed T-2 in the groups fed T-2 and MG at different time intervals are presented in Table 3. Progressive increase in T-2 adsorption may be due to the increased physical contact between the MG and T-2 as the time advanced. The results of the present study suggest that the MG can sequestrate up to 32% of T-2 by 120 min. *In vitro* binding assay by Dawson et al. (2001) and Raju and Devegowda (2002) corroborate our findings. These workers have demonstrated that the MG can sequestrate up to 33.4% of T-2.

It appears that there is no published information pertaining to *in vivo* binding ability of MG. However, previously *in vivo* binding trials have been conducted to study the ability of *Lactobacillus* and *Propionibacterium* strains (El-Nezami et al., 2000) and Hydrated sodium calcium aluminosilicate (Donald et al., 1987) to bind the aflatoxin in chicken GIT. *In vivo* binding assay employed in present study is similar to the intestinal loop technique followed by El-Nezami et al. (2000). From the results of this study, it is concluded that the MG can adsorb T-2 up to 32% in the GIT of chicken.

IMPLICATIONS

The *in vivo* binding studies on a toxin adsorbent reflects its actual toxin binding ability. Because, unlike *in vitro* studies where only pH is altered to simulate the gut environment, in case of *in vivo* studies a toxin adsorbent has to actively bind the toxin in the GIT where food material may hinder the formation of toxin-adsorbent complex. In addition, toxin present in GIT can escape from an adsorbent by moving through epithelial cell lining into the gastrointestinal system. Therefore, this *in vivo* method of determining the toxin binding has true potential to test the actual toxin adsorbing ability of an adsorbent. However, there is a lot of room to further improve on this method, say by adding an additional parameter like estimation of toxin present in the intestinal tissue that reflects the ability of an adsorbent to prevent toxin uptake by epithelial cells of the GIT.

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REFERENCES

Aravind, K. L., V. S. Patil, G. Devegowda, B. Umakantha and S. P. Ganpule. 2003. Efficacy of Esterified glucomannan to counteract Mycotoxicosis in naturally contaminated feed on

performance, serum biochemical and hematological parameters in broilers. Poult. Sci. (In press).

Burmeister, H. R. 1971. T-2 toxin production by *Fusarium tricinctum* on solid substrate. Appl. Microbiol. 21:739-742.

Chi, M. S., T. S. Robinson, C. J. Mirocha, S. P. Swanson and W. Shimoda. 1978. Excretion and tissue distribution of radioactivity from tritium labelled T-2 toxin in chicks. Toxicol. Appl. Pharmacol. 45:391-402.

Davidson, J. N., J. G. Babish and D. R. Delanely. 1987. Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin B_1 in chicken. Poult. Sci. 66(supp.1) 89(Abstr.).

Dawson, K. A., J. Evans and K. Manoj. 2001. Understanding the adsorption characteristics of yeast cell wall preparations associated with mycotoxin binding. In Biotechnology in the feed industry. (Ed. T. P. Lyons and K. A. Jacques). Nottingham University Press, Nottingham, UK, pp. 169-181.

Devegowda, G., M. V. L. N. Raju, N. Afzali and H. V. L. N. Swamy. 1998. Mycotoxin picture world wide: Novel solutions for their counteraction. In Biotechnology in the feed industry. (T. P. Lyons and K. A. Jacques). Nottingham University Press, Nottingham, UK, pp. 241-255.

Dvorska, J. E. and P. F. Surai. 2001. Effect of T-2 toxin, zeolite and mycosorb on antioxidant systems of growing quail. Asian-Aust. J. Anim. Sci. 14:1752-1757.

El-Nezami, H., H. Mykkanen, P. Kankaanpaa, S. Salminen and J. Ahokas. 2000. Ability of *Lactobacillus* and *Propionibacterium* strains to remove aflatoxin B_1 from the chicken duodenum. J. Food Prot. 63:549-552.

Feinberg, B. and C. S. Mc Laughlin. 1989. Biochemical mechanism of action of trichothecene mycotoxins. In Trichothecene Mycotoxins: Pathophysiologic Effects (I. V. R. Beasley). CRC Press, Boca Raton, pp. 27-35.

Giroir, L. E., G. W. Ivie and W. E. Huff. 1991. Comparative fate of the tritiated trichothecenes mycotoxin, T-2 toxin, in chickens and ducks. Poult. Sci. 70:1138-1143.

Graph Pad Prism Computer Programme. 1995. Version 2.00. Graph Pad Prism Software Inc., San Diego, USA.

Hoerr, F. J., W. W. Carlton and B. Yagen. 1981. The toxicity of T-2 toxin and diacetoxyscripenol in combination for broiler chickens. Food Cosmet. Toxicol. 19:185-188.

Lun, A. K., E. T. Moran, L. G. Young and E. G. McMillan. 1988. Disappearance of deoxynivalenol from digesta progressing along the chicken's gastrointestinal tract after intubating with feed containing contaminated corn. Bull. Environ. Contam. Toxicol. 40:317-324.

Manoj, K. B. and G. Devegowda. 2000. Efficacy of esterified glucomannan to ameliorate the toxic effects of T-2 toxin in laying hen. Poult. Sci. 79(Supp. 1) 270 (Abstr.).

Presluskuy, D. B., R. M. G. Hamilton, H. L. Trenholm and J. D. Miler. 1986. Tissue distribution and excretion of radioactive following administration of 14 C-labeled deoxynivalenol to White Leghorn hens. Fundam. Appl. Toxicol. 7:635-645.

Raju, M. V. L. N. and G. Devegowda. 2000. Influence of esterified glucomannan on performance and organ morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis (Aflatoxin, Ochratoxin and T-2 toxin). Br. Poult. Sci. 41:640-650.

Raju, M. V. L. N. and G. Devegowda. 2002. Esterified-Glucomannan in Broiler Chicken Diets-Contaminated with Aflatoxin, Ochratoxin and T-2 Toxin: Evaluation of its Binding Ability *(in vitro)* and Efficacy as Immunomodulator. Asian-Aust. J. Anim. Sci. 15:1051-1056.

Romer, T. R., T. M. Boling and J. L. Mac Donald. 1978. Gas liquid chromatographic determination of T-2 toxin and diacetoxyscirpenol in corn and mixed feeds. J. Assoc. Off. Anal. Chem. 61:801-807.

Rukmini, C. and R. V. Bhat. 1978. Occurrence of T-2 toxin in *Fusarium* infested sorghum from India. J. Agric. Food Chem. 26:647-649.

Ueno, Y., L. Ueno, Y. Litoi, Y. H. Tsunoda, M. Enomoto and K. Ohtsubo. 1971. Toxicological approaches to the metobolites of Fusaria. Jpn. J. Exp. Med. 41:521-525.

Yoshisawa, T., S. P. Swanson and C. J. Mirocha. 1980. In *vitro* metabolism of T-2 toxin in rats. Appl. Environ. Microbiol. 40:901.