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Effect of ASTRA-BEN 20® on Broiler Chicks Exposed to Aflatoxin B_1 or T2 Toxin[1,2]

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Abstract: The objectives of this study were: 1) to examine effects of a dietary sodium bentonite, ASTRA-BEN–20® (AB20) [Prince Agri Products, Inc., Quincy, Ill.], on in vitro binding of T2 toxin and aflatoxin B_1 (AFB1); and 2) to examine the effects of AB20 in broiler chicks fed 6 ppm dietary T2 toxin (T2) or 4 ppm aflatoxin B_1 (AFB1) to 21 d. Day-old male broiler chicks were randomly placed in batteries and assigned to one of nine dietary treatments (7 replicates of 5 chicks): control (C); 1% AB20; 2% AB20; T2; T2+ 1% AB20; T2+2% AB20; AFB1; AFB1+1% AB20; and AFB1+2% AB20. Individual BW, feed consumption by pen, period and cumulative feed conversions (PFC and CFC, respectively), and BW gains (BWG) were determined weekly. Liver weight (LW), relative liver weight (RLW), and liver lipid (%, PLL) were determined at d21 for AFB1 chicks. Oral lesions were scored on d21 for T2 chicks. One and 2% AB20 reduced BW during wk 1 and 2 compared to controls. AFB1 reduced BW during wk 2 and 3 and increased PLL at d21. Both 1 and 2% AB20 returned BW, BWG, and PLL to control levels. T2 reduced BW and BWG at wk 1, 2, and 3. T2 + 1% AB20 and T2 + 2% AB20 improved BW at wk 1 and 2 and BWG at wk 1 while T2 + 1% AB20 improved BW at wk 3 when compared to T2. T2 + 2% AB20 also decreased oral lesion severity compared to T2 at d21. PFC and CFC were increased by T2 and T2 + 1% AB20. CFC tended to improve when chicks were fed T2 + 2% AB20 ($P \leq 0.06$). AB20 protected broiler chicks from dietary AFB_1 and provided some protection against dietary T2.

Key words: T2 toxin, aflatoxin B1, sodium bentonite, ASTRA-BEN–20®

INTRODUCTION

The *in vitro* and *in vivo* adsorption of aflatoxins by bentonite clays (montmorillonite) is well documented (Kubena *et al*., 1990; Phillips *et al*., 1994; Taylor, 1999). *In vitro* adsorption by bentonite has been used experimentally to remove aflatoxin M1 from milk (Applebaum and Marth, 1981). Moreover, *in vivo* adsorption of aflatoxins by bentonite included in contaminated feed has been repeatedly demonstrated to protect poultry, swine, sheep, and cattle from the toxic effects of aflatoxin and to reduce the transmission of aflatoxin M1 into milk (Phillips *et al*., 1994; Diaz *et al*., 2004: Miazzo *et al*., 2005). The aflatoxin/clay complex is believed to pass intact through the digestive system of the animal. Most of these clay products are Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (2004). Complicating factors in interpreting the scientific and popular press literature in regards to clay products are: 1) the complex nomenclature and the variable chemical and physical nature of the clays tested; and 2) the use of *in vitro* data to imply *in vivo* efficacy. While *in vitro* experiments have revealed binding between clays and mycotoxins including aflatoxin B_1, *in vivo* studies have not identified the amount of dietary clay that offer consistent protection to poultry (Desheng *et al*., 2005). Conflicting results have been reported in regards to dietary concentrations of activated charcoal preparations tested for protection against mycotoxins (Piva *et al*., 2005; Kubena *et al*., 1990; Edrington *et al*., 1997). The objectives of this study were to determine if ASTRA-BEN–20® (AB20) at 1 and 2% of diet would cause *in vitro* binding of T2 toxin and aflatoxin B1as well as *in vivo* protection of broiler chicks from the effects of dietary T2 toxin and aflatoxin B1.

MATERIALS AND METHODS

***In vitro* toxin binding assay:** ASTRA-BEN-20® (Prince Agri Products, Inc., Quincy, Ill.) was tested for *in vitro* binding of AFB1 (10µg/mL) or T2 toxin (10µg/mL) as a 2% suspension in 10% methanol at pH 3, 7, and 10.1 (the pH of the 2% AB suspension). The pHs of the suspensions were adjusted with 2N sodium hydroxide and 2N hydrochloric acid. Suspensions were maintained by a magnetic stirring bar at room temperature for 1hr. After incubation, the AB20 was removed as a solid pellet after centrifugation at 1500rpm for 15 min in a table-top centrifuge. The supernatants were analyzed for unbound AFB1 by HPLC with fluorescence detection of the bromine derivative (Traag *et al*., 1987) and for unbound T2 toxin by gas-liquid chromatography with electron capture detection of the trimethylsilyl derivative (Kamimura *et al*., 1981; Rizzo *et al*., 1986). A model GC-14A[1] was operated under the following conditions: nitrogen carrier gas at 4mL/min; injector temperature 250°C; detector temperature 300°C; temperature program at 185-260°C at 4°C/min. Analysis was performed on a 30-m Megabore (.53mm ID) DB-1701 fused silica column.

Table 1: Composition of the diet

Ingredient	Percent
Corn	53.67
Soymeal	32.00
Limestone	1.00
Poultry fat	5.00
Dical 18.5 Phosphate	1.30
Poultry meal	6.00
DL Methionine	0.22
Lysine	0.10
Salt	0.24
Sodium Bicarbonate	0.30
Minerals[A]	0.03
Vitamins[B]	0.05
Selenium[C]	0.06
Ethoxyquin	0.01
Manganese Sulfate	0.02
Calculated Analysis	
Protein	23.6
ME (kcal/kg)	3150
Crude Fat	8.1
Methionine	0.62
TSAA	1.01
Lysine	1.39
Calcium	0.95
Available Phosphorus	0.49

[A]Minerals mix supplied the following per kg of diet: 120 mg Zn as $ZnSO_4$ H_2O; 120 mg Mn as $MnSO_4$ H_2O; 80 mg Fe as $FeSO_4$ H_2O; 10 mg Cu as $CuSO_4$; 2.5 mg I as Cu $(IO_3)_2$; 1.0 mg Co as $CoSO_4$. [B]Vitamin mix supplied the following per kg of diet when added at 0.2: vitamin A, 6,600 IU; vitamin D_3, 2000 ICU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin , 6.6 mg; niacin, 55mg; d-pantothenate, 11mg menadione, 2mg; folic acid, 1.1mg; thiamine, 2mg; pyridoxine, 4mg; d-biotin, 126µg; ethoxyquin, 50mg. [C]Selenium premix supplied .21mg Se, as Na_2SeO_3.

Dietary sodium bentonite and toxins: ASTRA-BEN-20® was added at a rate of 1% and 2% to the feed on a wet weight basis. Crystalline T2 toxin was prepared and checked for purity according to Richardson (1986). T2 was added to T2 treatment diets at 6 ppm, which has been shown to depress growth in chickens and induce oral lesions in chicks (Edrington *et al*., 1997; Sklan *et al*., 2001). Aflatoxin B1 (AFB1) was added to AFB1 treatment diets at 4 ppm which is within a concentration range previously demonstrated to depress growth in the chick (Kubena *et al*., 1990; Scheideler, 1993; Rafai *et al*., 2000; Sklan *et al*., 2001).

Dietary treatments: Standard broiler starter diet was fed throughout the study (Table 1). It was analyzed for AFB1, T2, deoxynivalenol, zearalenone, and fumonisins prior to use. Pure crystalline T2 or AFB1 was added to diets by dissolving each toxin in acetone; solutions were mixed with 2.3 kg of feed and allowed to dry in a fume hood. The toxin-premix was added per 45.5 kg of feed. Nine dietary treatments were randomly assigned to 7 replicate pens per treatment: control (C); 1% AB20; 2% AB20; T2; T2+ 1% AB20; T2+2% AB20; AFB1; AFB1+ 1% AB20; and AFB1+ 2% AB20.

Experimental chicks and measurements: Day-old male broiler chicks (Arbor Acres X Yield Master; n=325) hatched from the North Carolina State University broiler breeder flock were maintained under conditions approved by the North Carolina State University Institutional Animal Care and Use Committee. Broilers were housed in electrically heated batteries under a 23L:1D lighting schedule for 21 d and feed and water were available ad libitum. The birds were weighed, banded, and assigned randomly to pens containing 5 chicks per pen. Weekly individual BW and feed consumption by pen were measured. Period (PFC) and cumulative (CFC) feed conversions were calculated. D21 oral lesion scores were assigned for T2 toxin treatments and d21 liver weights and percent liver lipid were determined for aflatoxin treatments (Smith and Hamilton, 1970).

Experimental design and statistical analysis: A completely randomized experimental design was used to determine the effects of dietary sodium bentonite, AB20, on broiler chicks fed diets with 6 ppm T2 or 4 ppm AFB1 to 21d. There were 9 treatments with 7 pens of 5 chicks per dietary treatment.

Regression analysis of SAS® (1989) was used to determine the effects of treatments on performance parameters. Treatment means were separated using Least Square Means. Treatment means were considered significant at $P \leq 0.05$.

RESULTS

***In vitro* binding experiments:** The *in vitro* binding of T2 and AFB1 by AB20 is presented in Table 2. The AB20 bound the most T2 toxin at a pH of 10.1 (74.4%), the normal pH for the additive in 10% methanol solution. ASTRA-BEN 20® bound the greatest percentage of AFB1 at pH 3 (99.5%), but bound at least 97% all pH values tested.

Comparison of control diets: Dietary AB20 at both 1% and 2% decreased BW and BWG at wks 1 and 2. During wk 3, dietary AB20 decreased BW and BWG at the 1% level when compared to the control diet (Table 3). Cumulative feed conversions were significantly different among the control feeds for the 0-3 wk period only, with chicks fed the control diet having better CFC than chicks fed 1% AB20 (1.43 vs. 1.62), with chicks fed 2% AB20 having intermediate CFC (1.49).

Comparison of dietary aflatoxin B1 treatments: Chicks fed the AFB1 diet had significantly lower BW and BWG at wks 2 and 3 compared to the control and AFB1+1% AB20 or AFB1+2% AB20 diets (Table 4). Overall BWG of chicks fed AFB1 was significantly lower than the remaining treatments and the control diet (data not shown). Chicks fed the AFB1 diet had significantly

Table 2: Percent (%) of added mycotoxin bound and removed by 2% suspension of ASTRA-BEN-20® from solution in 10% aqueous methanol

Toxin	pH 3	pH 7	pH 10.1
T2 toxin	20.4	25.2	74.4
Aflatoxin B1	99.5	98.2	97.0

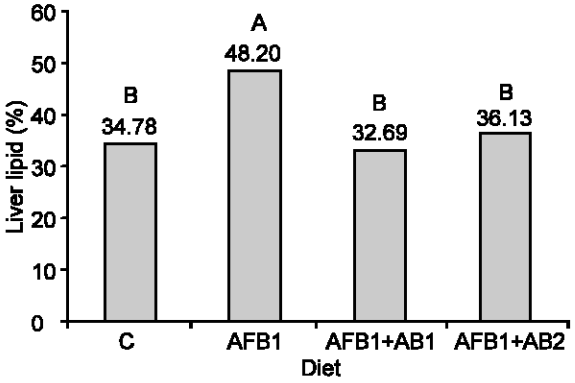

Fig. 1: Effect of ASTRA-BEN-20® at 1% (AB1) and 2% (AB2) on percent liver lipid of broiler chicks fed a diet with 4ppm aflatoxin B1 (AFB1) when compared to chicks fed a control diet (C) at d21. Bars with different letters are statistically significant (P≤0.01) from one another.

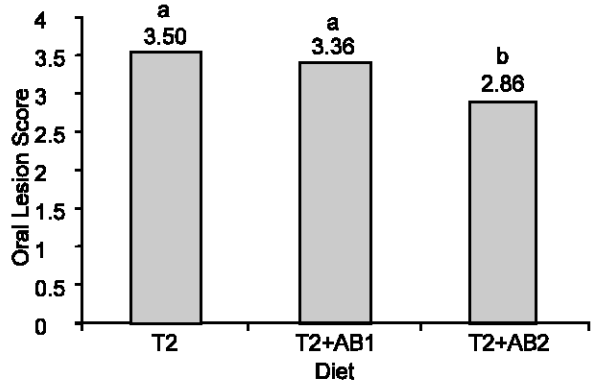

Fig. 2: Effect of ASTRA-BEN-20® at 1% (AB1) and 2% (AB2) on oral lesion scores of broiler chicks fed a diet with 6 ppm T2 toxin (T2) when compared to chicks fed a control diet (C) at d21. Bars with different letters are statistically significant (P≤0.05) from one another.

higher PLL than chicks fed the control or AFB1+1 and 2% AB20 diets (Fig. 1).

Comparison of dietary T2 treatments: Body weights of chicks fed T2 were significantly lower (p< 0.05) than the control, T2+1% AB20, and T2+2% AB20 diets at wks 1 and 2 (Table 5). By wk 3, T2 chicks had lower BW than chicks fed the control and T2+1% AB20 diets, but no longer had lower BW than the T2+2% AB20 diet (Table

Table 3: Effect of ASTRA-BEN-20® at 1% (AB1) or 2% (AB2) on body weight and body weight gain of broiler chicks fed a control diet

	Body Weight		
	Week		
Diet	1	2	3
Control	159.8[a]	436.0[a]	813.4[a]
Control + AB1	151.1[b]	413.6[b]	742.1[b]
Control + AB2	153.3[b]	416.4[b]	789.5[a]
	Body Weight Gain		
Control	120.2[a]	276.1[a]	377.4[a]
Control + AB1	111.2[b]	262.2[b]	328.5[b]
Control + AB2	113.6[b]	263.3[b]	373.2[a]

[a,b]Means within a column lacking a common superscript differ (P ≤ 0.05).

Table 4: Effect of ASTRA-BEN-20® at 1% (AB1) or 2% (AB2) on body weight and body weight gain of broiler chicks fed a diet containing 4ppm aflatoxin B1 (B1)

	Body Weight		
	Week		
Diet	1	2	3
Control	160.0	435.2[a]	810.8[a]
B1	161.1	392.4[b]	682.7[b]
B1 + AB1	159.7	433.7[a]	816.4[a]
B1 + AB2	157.7	431.5[a]	812.7[a]
	Body Weight Gain		
Control	120.3	275.2[a]	375.7[a]
B1	120.7	230.9[b]	290.3[b]
B1 + AB1	119.2	274.4[a]	382.7[a]
B1 + AB2	116.7	273.7[a]	381.2[a]

[a,b]Means within a column lacking a common superscript differ (P ≤ 0.05).

Table 5: Effect of ASTRA-BEN-20® at 1% (AB1) or 2% (AB2) on body weight and body weight gain of broiler chicks fed a diet containing 6ppm T2 toxin (T2)

	Body Weight		
	Week		
Diet	1	2	3
Control	160.0[a]	435.9[a]	812.3[a]
T2	139.8[c]	351.8[c]	657.2[c]
T2 + AB1	151.3[b]	371.0[b]	694.8[b]
T2 + AB2	155.4[b]	379.0[b]	692.7[b c]
	Body Weight Gain		
Control	120.3[a]	275.9[a]	376.4[a]
T2	98.7[c]	212.0[b]	305.5[b]
T2 + AB1	110.8[b]	219.7[b]	323.8[b]
T2 + AB2	115.2[ab]	223.7[b]	313.7[b]

[a,b]Means within a column lacking a common superscript differ (P ≤ 0.05)

5). Chicks fed T2 toxin had lower BWG than chicks fed any other diet during wk 1 (Table 5). During wks 2 and 3, all chicks on diets with T2 toxin added had lower BWG than chicks fed the control diet (Table 5). Control chicks had higher overall BWG than chicks fed the T2+AB20

diets (772g vs. 653g), which had higher BWG than T2 chicks (653g vs. 616g). Two-wk CFC was better in control chicks than in any other diet (1.20 vs. 1.37). Three-wk CFC was better in control chicks than in chicks fed the T2 and T2+1% AB20 diets (1.42 vs. 1.72 and 1.62, respectively), with chicks fed the T2+2% AB20 diet having intermediate 3-wk CFC to all other diets (1.57). Oral lesion scores were significantly reduced in chicks fed the T2+2% AB20 diet (Fig. 2).

DISCUSSION

This study demonstrated the protection of broiler chicks against the effects of T2 toxin using sodium bentonite clay. Carson and Smith (1983) have previously reported that the feeding of bentonite to rats decreased the toxicity of T2. In the present study, the body (Table 5) weights of broilers fed 1% AB20 decreased 15% ($p < 0.05$) compared to controls while the fed T2 alone decreased 19% ($p < 0.05$). Body weight gain, however decreased ($p < 0.05$) similarly for T2 and T2 plus 1 or 2% AB20. Two percent AB20 however, decreased oral lesion scares $p < 0.05$ in broilers by 18% compared to controls. Hence, it is our opinion that the protective effects of AB20 against T2 toxins in broilers, although not complete, represented a modest but significant attenuation of the effects of the toxin on broilers. Similar partial protection of broilers against aflatoxin contaminated feed by sodium bentonite was reported by Miazzo *et al*. (2005). While the protection afforded by AB20 against T2 toxin was moderate, the experimental design used in the present study made our test of the concept very conservative. A very high concentration of T2 toxin was used (4µg/kg) which is much higher than that previously reported. Indeed, Carson and Smith (1983) reported that bentonite fed at 10% of the diet was the most effective treatment at overcoming feed refusal and growth depression in male rats fed 3µg T2/g of feed.

Other factors could influence the binding of T2 toxin removal to bentonite clays. In the present study, the in vitro binding of T2 toxin to a 2% AB20 10% methanol (w/v) solution increased 265% at pH 10.1. One must be cautious however, in interpreting this increase as T2 binding. The high pH of the AB20/10% methanol solution may be sufficient to partially hydrolyze the functional moities in T2 toxin. Additionally, pH10 is outside a physiological range. The high pH of AB20(10.1) while suspended in 10% methanol may be sufficient to partially hydrolyze the functional acetyl and isovaleryl groups in T2.

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