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Effect of Sodium Bentonite in Broiler Chickens Fed Diets Contaminated with Aflatoxin B_1

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Abstract: In an *in vivo* study, Sodium Bentonite (SB) was evaluated for its ability to reduce the deleterious effects of Aflatoxin B_1 (AFB_1) in broiler chickens. In this study, 288 days old Ross male broiler chickens were used and randomly assigned to 9 treatment groups, with 4 replicates of 8 birds each. Three levels of Aflatoxin B_1, AFB_1 (0, 500 and 1000 ppb) and 3 levels of SB (0.0, 0.5 and 1.0%) were fed to chickens from 0-42 days of age. Feeding levels of AFB_1 alone significantly decreased ($p<0.05$) feed intake, body weight and body weight gain, while, SB levels alone or in combination with AFB_1, had no effect on the mentioned traits. No significant difference was seen for feed conversion ratio during periods of 0-42 days of age. In the diet containing AFB_1, relative weight of the carcass, thigh and breast were significantly decreased ($p<0.05$). Adding AFB_1 alone into the diets significantly increased ($p<0.05$) serum ALT, AST and LDH enzymes and decreased serum GGT enzyme ($p<0.05$). However, SB alone or in combination with AFB_1 had no effect on the above mentioned enzymes. It is suggested that sodium bentonite at the levels of 0.5-1.0% might be used for reducing the adverse effects of aflatoxins in broiler chickens.

Key words: Aflatoxin B_1, sodium bentonite, performance, blood enzymes, broilers

INTRODUCTION

Mycotoxins are secondary toxic metabolites formed by certain fungi growing on Food and animal feeds usually belonging to the genus of *Aspergillus flavus* and *Aspergillus parasiticus* (Tedesco *et al*., 2004) and can happen as natural contaminants of poultry feed (Leeson *et al*., 1995). Contamination of cereals and grains and their related products with mycotoxins causes food and feed-born intoxicants in human and animals (Meltor, 2001). Aflatoxin B_1 (AFB_1) is the most potent mycotoxins causes a wide range of clinical and sub-clinical problems in poultry (Kermanshahi *et al*., 2007; Hussein and Brasel, 2001). Many different toxic effects for aflatoxins, including reduced performance, hepatic intoxication, adverse effects on carcass as well as egg shell quality, immunosuppression and carcinogenicity have been reported in poultry (Charmley *et al*., 1995). Increase in relative weights of liver, kidney, heart, proventriculus, gizzard, spleen and pancreas in broilers consuming aflatoxin contaminated diets, have been reported (Kubena *et al*., 1990). Liver enlargement and discoloration in broilers have been reported by Kermanshahi *et al*. (2007). Also, it has been demonstrated that aflatoxins are able to alter concentrations of some blood enzymes (Kubena *et al*., 1990). Negative effects of aflatoxins in poultry production are both dose and time dependent (Leeson *et al*., 1995). At present, aflatoxins are considered unavoidable contaminants of feed and food. To combat animal aflatoxicosis and detoxification of mycotoxin-contaminated feeds, different physical methods are recommended but since mycotoxins are heat stable and resistance to heat, current agricultural practices and routine feed processing procedures can not reduce their toxicity (Bassapa and Shanta, 1996; Doyle *et al*., 1982). In animal nutrition, the use of mycotoxin adsorbents as feed additives is one of the most promising and widely used approaches to reduce the deleterious effect of aflatoxicosis in farm animals. It is good method to reduce carry-over of mycotoxins from the contaminated feeds into animal-derived products (Ramos *et al*., 1996). Phillips *et al*. (1987, 1988) in *in vitro* and *in vivo* studies reported that Hydrated Sodium Calcium Aluminosilicate (HSCAS) have the high capacity to adsorb aflatoxins in poultry HSCAS is a generic description and it does not uniquely define the material

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of use. Most of the classified HSCAS are as natural montmorillonits and bentonits (Ramos and Fernadez, 1997). In many reports, different adsorbents including zeolites, acticated charcoal and phylosilicate minerals have been used to decrease the negative effect of aflatoxins (Devegowda *et al*., 1996; Rosa *et al*., 2001). There are many adsorbents in the markets and their capacity to adsorb specific mycotoxins are not clear. Although, from nutritional point of view, bentonite type of binders would supply little nutritive value but, it is the most potent mineral substances to reduce the deleterious effect of aflatoxins in poultry (Miazzo *et al*., 2000, 2005; Rosa *et al*., 2001). Therefore, the purpose of this research was to evaluate the capacity of locally available bentonits in male broiler chickens fed diets contaminated with aflatoxin B_1.

MATERIALS AND METHODS

Total 288 days old Ross male broiler chickens were divided into 9 treatments of 4 replicates each and randomly assigned to each of 36 floor pens of 1×1 m. All birds had free access to feed and water during the experiment (0-42 days). Aflatoxin B_1 (AFB_1) for this experiment was produced using *Aspergillus parasiticus* strain 2999 according to the method described by Shotwell *et al*. (1966) and West *et al*. (1973). More than 80% of the aflatoxin produced by this strain is B_1 type (Rosa *et al*., 2001). AFB_1 concentration was determined using TLC according to Afzali (1998) method. Relative weights of edible meat, thigh and breast to live body weight measured at 42 days of age by killing 2 birds from each replicate of treatments. Serum concentrations of Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Gama Glutamyl Transferase (GGT) and Lactate Dehydrogenase (LDH) were determined at 21 and 42 days of age using commercial available kits (Darman Kave Research Laboratory, Isfahan, Iran). Starter, grower and finisher diets (Table 1) were formulated for 0-14, 14-28 and 28-42 days of age, respectively, according to the Ross 308 manual recommendations. Dietary treatments were consisted of 3 levels of AFB_1 in diet (0.0, 500 and 1000 ppb) and 3 levels of a locally prepared sodium bentonite (0.0, 0.5 and 1.0% SB, Ashkaftook mine, South of Khorasan, Iran). The SB composition was SiO_2,58.80%; Al_2O_3, 18.50%; Fe_2O_3, 5.0%; CaO, 2.15%; MgO, 2.10%; Na_2O, 3.50%; K_2O, 0.45%; TiO_2, 0.15%; moisture, 9.35%. In order to reach intended AFB_1 concentrations in diets, an appropriate mixture of *Aspergillus parasiticus* culture on rice was added into the basal diet. AFB_1 contaminated diets were fed to broiler chickens from 0-42 days of age. Feed intake and body weight gain recorded weekly and then performance data expressed as week basis.

Table 1: Composition of the experimental diets

Ingredients (%)	Starter (0-14 days)	Grower (14-28 days)	Finisher (28-42days)
Yellow corn	59.97	60.38	64.65
Soybean meal (44%)	30.00	30.00	28.13
Fish meal	6.20	2.50	0.00
Veg. oil	3.46	3.51	3.30
Dicalcium phosphate	1.40	1.46	1.71
Oyster shell	1.10	1.14	1.25
Vitamin premix[2]	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25
Salt	0.25	0.25	0.25
DL-Methionine	0.12	0.19	0.16
L-Lysine	0.00	0.07	0.05
Sodium Bentonite (SB)[1]	0.00	0.00	0.00
Aflatoxin B_1 (ppb) (AFB_1)	0.00	0.00	0.00
Total	100.00	100.00	100.00
Calculated composition			
Metabolic energy (kcal kg^{-1})	3000.00	3000.00	3000.00
Crude protein (%)	22.00	20.00	18.00
Calcium (%)	1.00	0.90	0.90
Available phosphorus (%)	0.50	0.45	0.45
Lysine (%)	1.20	1.09	0.92
Methionine + cystine (%)	0.81	0.81	0.72
Tryptophan (%)	0.22	0.20	0.18

Arrangements of 9 treatments in each period (AFB_1 (ppb))

SB (%)	0	500	1000
0	0.0 + 0	0.0 + 500	0.0 + 1000
0.5	0.5 + 0	0.5 + 500	0.5 + 1000
1.0	1.0 + 0	1.0 + 500	1.0 + 1000

[1]In each period, 9 treatment diets prepared as arranged in treatment arrangements, [2]Each kg of vitamin premix contained: vitamin A, 3,600,000 IU; vitamin D_3, 800,000 IU; vitamin E, 7,200 IU; vitamin K_3, 800 mg, vitamin B_1, 720 mg; vitamin B_2, 2,640 mg, vitamin B_3, 4,000 mg, vitamin B_5, 12,000 mg, vitamin B_6, 1,200 mg vitamin B_9, 400 mg; vitamin B_{12}, 6 mg, vitamin H_2, 40 mg, choline chloride, 200,000 mg, [3]Each kg of mineral premix contained: Mn, 40,000 mg, Fe, 20,000 mg, Zn, 40,000 mg; Cu, 4,000 mg, Se, 80 mg

Statistical analysis: The 9 treatments were allotted in a completely randomized design, with 3×3 factorial arrangements. All data were analyzed using GLM procedure of SAS and treatment means were compared by Touki test (SAS, 1990).

RESULTS AND DISCUSSION

Appearance and performance: In the 1st and 2nd week of experiment, chickens didn't show any sign of toxicity in their appearance. Gradually, at week 3 and later on, chickens fed AFB_1 showed some sign of aggressions, pale color in their combs and skins, fluffy feathers and retarded growth rate. Chickens receiving SB or SB with AFB_1 had normal appearance.

Data of weekly Feed Intake (FI) is presented in Table 2. Treatments had no effect on Feed intake till the end of week 3. However, chickens receiving AFB_1 alone (500 and 1000 ppb) fed lower feed when compared to other treatments. At week 4-6 FI significantly decreased in birds fed AFB_1 contaminated diets ($p<0.05$). The lowest FI was seen in birds fed 1000 ppb AFB_1 alone. However, the difference between 500 and 1000 ppb AFB_1 was not

Table 2: Effect of treatments on feed intake of broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		Experimental periods (weeks)					
AFB_1 (ppb)	SB (%)	1	2	3	4	5	6
0	0	127.7	273.4	507.6	753.3^{a}	861.8^{a}	1051.1^{a}
500	0	125.1	255.1	500.3	598.7^{b}	716.9^{b}	740.50^{b}
1000	0	124.2	246.7	496.6	589.8^{b}	665.1^{c}	663.90^{c}
0	0.5	129.2	276.3	507.4	751.9^{a}	880.6^{a}	1075.4^{a}
500	0.5	130.7	276.5	521.6	758.9^{a}	871.6^{a}	1056.7^{a}
1000	0.5	128.7	277.5	502.7	752.3^{a}	875.8^{a}	1058.8^{a}
0	1	133.8	278.6	514.4	741.3^{a}	882.3^{a}	1081.3^{a}
500	1	130.6	270.6	515.8	735.4^{a}	885.3^{a}	1060.9^{a}
1000	1	130.7	274.2	519.9	759.8^{a}	872.2^{a}	1068.4^{a}

[1]AFB_1: Aflatoxin B_1; SB: Sodium Bentonite; Results for each treatments are obtained from 32 chickens in each treatment with ±SEM = 11.7; $^{a, b, c}$In each column, means with different superscripts are significantly different ($p<0.05$)

Table 3: Effect of treatments on body weight gain of broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		Experimental periods (weeks)					
AFB_1 (ppb)	SB (%)	1	2	3	4	5	6
0	0	95.0	190.1	368.0^{a}	504.7^{a}	459.2^{a}	520.6^{a}
500	0	91.9	170.0	337.8^{ab}	355.8^{b}	365.6^{b}	347.5^{b}
1000	0	89.5	166.3	323.5^{b}	349.6^{b}	327.3^{b}	304.4^{c}
0	0.5	97.9	196.7	368.3^{a}	500.4^{a}	478.4^{a}	521.9^{a}
500	0.5	96.0	194.8	366.1^{a}	498.6^{a}	458.1^{a}	520.0^{a}
1000	0.5	95.2	192.4	366.3^{a}	486.9^{a}	472.3^{a}	510.2^{a}
0	1	97.3	195.6	369.1^{a}	480.9^{a}	475.0^{a}	523.6^{a}
500	1	95.2	185.1	369.4^{a}	476.6^{a}	478.8^{a}	520.8^{a}
1000	1	95.0	188.3	364.4^{a}	468.9^{a}	463.4^{a}	520.5^{a}

[1]AFB_1: Aflatoxin B_1, SB: Sodium Bentonite, Results for each treatments are obtained from 32 chickens in each treatment with ±SEM = 14.5, $^{a, b, c}$In each column, means with different superscripts are significantly different ($p<0.05$)

different only at 4th week. Addition of SB into contaminated diets increased FI similar to control birds. Data indicated that by increasing levels of AFB_1, FI decreases more and SB addition alleviated this effect. These results are in agreement with those of Ledoux *et al.* (1999), Santin *et al.* (2003), Kermanshahi *et al.* (2007), Rosa *et al.* (2001) and Miazzo *et al.* (2005). Data of weekly Mean Body Weight (MBW) and weekly Body Weight Gain (BWG) of treatments are presented in Table 3 and 4. The results and trends were similar with those of FI. Mean body weight and body weight gain of chickens fed AFB_1 alone started to decrease from 3rd week and continued to end of the experiment. The deleterious effect of AFB_1 was also changed to normal conditions (control) when SB added into their contaminated diets. The results of MBW and BWG in this experiment are also in agreement with those of other studies (Rosa *et al.*, 2001; Desheng *et al.*, 2005; Miazzo *et al.*, 2005; Kermanshahi *et al.*, 2007). Dersjant-Li *et al.* (2003) reviewed the impact of low oncentrations of aflatoxins in poultry diets and suggested that the growth reduction due to aflatoxin contaminated diets can be related to reduction in both feed intake and feed efficiency. In this experiment, AFB_1 did not negatively influence feed conversion ratio (Table 5) that

Table 4: Effect of treatments on mean body weight of broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		Experimental periods (weeks)					
AFB_1 (ppb)	SB (%)	1	2	3	4	5	6
0	0	141.8	331.8	699.9^{a}	1204.5^{a}	1663.8^{a}	2184.4^{a}
500	0	139.0	308.9	646.8^{ab}	1002.5^{b}	1368.1^{b}	1715.6^{b}
1000	0	135.9	302.2	625.7^{b}	975.30^{b}	1302.7^{b}	1607.0^{c}
0	0.5	144.6	341.3	709.6^{a}	1210.0^{a}	1688.4^{a}	2210.3^{a}
500	0.5	143.7	338.5	704.6^{a}	1203.1^{a}	1661.3^{a}	2181.3^{a}
1000	0.5	142.3	334.8	701.1^{a}	1188.0^{a}	1660.3^{a}	2170.5^{a}
0	1	144.2	339.8	708.9^{a}	1189.8^{a}	1664.8^{a}	2188.4^{a}
500	1	142.8	327.9	697.3^{a}	1173.9^{a}	1652.7^{a}	2173.4^{a}
1000	1	141.5	329.8	694.3^{a}	1163.1^{a}	1626.6^{a}	2147.0^{a}

[1]AFB_1: Aflatoxin B_1; SB: Sodium Bentonite; Results for each treatments are obtained from 32 chickens in each treatment with ±SEM = 24.5; $^{a, b, c}$In each column, means with different superscripts are significantly different ($p<0.05$)

Table 5: Effect of treatments on Feed Conversion Ratio (FCR) of broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		Experimental periods (weeks)					
AFB_1 (ppb)	SB (%)	1	2	3	4	5	6
0	0	1.34	1.44	1.38	1.51	1.94	2.02
500	0	1.36	1.51	1.48	1.69	1.96	2.16
1000	0	1.39	1.49	1.56	1.69	2.03	2.18
0	0.5	1.32	1.41	1.40	1.51	1.86	2.06
500	0.5	1.37	1.42	1.43	1.52	1.91	2.03
1000	0.5	1.35	1.45	1.38	1.55	1.87	2.08
0	1	1.38	1.44	1.39	1.55	1.88	2.07
500	1	1.37	1.47	1.41	1.54	1.87	2.05
1000	1	1.38	1.46	1.44	1.63	1.89	2.07

[1]AFB_1: Aflatoxin B_1; SB: Sodium Bentonite; Results for each treatments are obtained from 32 chickens in each treatment with ±SEM = 0.07; In each column, means with no superscripts are not significantly different ($p>0.05$)

Table 6: Effect of treatments on relative organ weights (g/100 g of body weight) of broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		Relative organ weights (%)		
AFB_1 (ppb)	SB (%)	Carcass weight	Thigh	Breast
0	0	73.460^{a}	22.250^{a}	22.840^{a}
500	0	67.410^{b}	19.890^{b}	19.660^{b}
1000	0	66.540^{b}	18.840^{b}	18.240^{b}
0	0.5	72.120^{a}	22.040^{a}	21.820^{a}
500	0.5	71.920^{a}	22.010^{a}	21.950^{a}
1000	0.5	71.080^{a}	21.970^{a}	21.810^{a}
0	1	71.720^{a}	22.030^{a}	22.200^{a}
500	1	71.500^{a}	21.960^{a}	21.770^{a}
1000	1	71.310^{a}	21.280^{a}	21.540^{a}
±SEM		1.040	0.410	0.600

[1]AFB_1, aflatoxin B_1; SB, Sodium Bentonite, $^{a, b, c}$In each column, means with different superscripts are significantly different ($p<0.05$)

is in agreement with the results of Edrington *et al.* (1997). Feed Conversion Ratio (FCR) is an index of feed intake and body weight gain and usually wouldn't be considered alone.

Relative organ weights: The effect of AFB_1 alone or in combination with SB on carcass weight, thigh and breast yield relative to live body weight is presented in Table 6. The data clearly shows the negative effect of AFB_1 on the

Table 7: Serum ALT, AST, GGT and LDH concentrations (U/L) in broiler chickens fed diets containing different levels of aflatoxin B_1 and sodium bentonite

Treatments[1]		ALT[2] (day)		AST (day)		GGT (day)		LDH (day)	
AFB_1 (ppb)	SB (%)	21	42	21	42	21	42	21	42
0	0	57.130^{a}	45.000^{a}	139.250^{a}	109.500^{a}	32.55^{a}	35.59^{a}	318.25^{a}	314.00^{a}
500	0	62.630^{ab}	55.000^{bc}	155.630^{bc}	124.130^{b}	24.58^{b}	26.88^{b}	362.88^{ab}	376.38^{b}
1000	0	68.130^{b}	60.880^{b}	163.130^{c}	135.250^{c}	20.63^{b}	21.50^{c}	391.50^{b}	432.75^{c}
0	0.5	57.630^{a}	46.130^{a}	140.500^{a}	110.880^{a}	32.13^{a}	34.38^{a}	319.00^{a}	317.00^{a}
500	0.5	60.380^{a}	49.250^{ac}	143.380^{a}	114.500^{a}	30.88^{a}	33.46^{a}	326.00^{a}	322.00^{a}
1000	0.5	59.630^{a}	50.380^{ac}	146.630^{ab}	118.250^{ab}	31.00^{a}	32.63^{a}	332.75^{a}	329.25^{ab}
0	1	57.880^{a}	48.500^{ac}	142.000^{a}	112.880^{a}	31.25^{a}	33.75^{a}	321.00^{a}	318.00^{a}
500	1	59.130^{a}	48.750^{ac}	143.630^{a}	116.750^{ab}	31.19^{a}	33.25^{a}	325.13^{a}	324.25^{ab}
1000	1	58.880^{a}	49.750^{ac}	147.250^{ab}	115.880^{ab}	31.80^{a}	32.56^{a}	336.88^{a}	326.13^{ab}
±SEM	-	2.450	3.3700	1.480	18.580	-	-	-	-

[1]AFB_1: Aflatoxin B_1, SB: Sodium Bentonite, Results for each treatments are obtained from 32 chickens in each treatment, [2]ALT: Alanine Aminotransferase, AST: Aspartate Aminotrasferase, GGT: Gamma Glutamyl Trasferase, LDH: Lactate Dehydrogenase, [a, b, c]In each column, means with different superscripts are significantly different ($p<0.05$)

mentioned organ weights. AFB_1 significantly decreased carcass weight, thigh and breast yield ($p<0.05$). This negative effect was cleared when SB added as feed supplement into the experimental diets. Kermanshahi *et al.* (2007) showed that AFB_1 significantly increases some of the organ weights. Liver is the first organ affected by AFB_1 (Leeson *et al*., 1995). Increase in liver relative weight has also been reported by Kubena *et al.* (1993) during aflatoxicosis. This enhancement in liver weight is usually due to fat deposition in the liver (Leeson *et al*., 1995). Increase in internal organ weights like liver may decreases relative carcass weight. As seen in this study, the lower values of edible parts of the carcass (most valued parts) brings lots of losses to poultry producers and using SB is one of the economical ways to combat with this problem.

Blood enzymes: Serum concentrations of ALT, AST, GGT and LDH for different treatment groups are shown in Table 7. AFB_1 alone in the diet at both 21 and 42 days of age caused a significant increase in serum concentrations of ALT and AST ($p<0.05$).

The results obtained in this study concerning increase activity of ALT and AST when chickens fed AFB_1 are in agreement with those of many studies (Devegowda *et al*., 1996; Celik *et al*., 2005; Kermanshahi *et al*., 2007). Dafalla *et al*. (1987) also reported an increase in serum concentration of AST and ALT due to AFB_1 administration into the diet. Generally, AST and ALT are intracellular enzymes that do not belong to plasma; so, their appearance in serum indicates cell injury (Coles, 1974).

Increase in serum concentrations of AST and ALT in this experiment, might be a result of hepatocytes injury. Using bentonite brought the ALT and AST enzyme activities back to normal conditions that is also showed by Eraslan *et al*. (2006). AFB_1 alone significantly increased GGT activity in both 21 and 42 days of age ($p<0.05$).

The lowest level of GGT was seen in chickens fed 1000 ppb AFB_1. Supplementation of contaminated diets with SB corrected the GGT values similar to that of control treatment in both ages. Our results concerning decrease in GGT activity and AFB_1 effect is similar to those of Jansen *et al.* (2006) and Ersalan *et al.* (2006). Eraslan *et al.* (2006) was also shown that using SB alleviates the effect of AFB_1 on GGT and increases its activity. AFB_1 significantly increased activity of LDH in both 21 and 42 days of age ($p<0.05$).

This effect was more pronounced in 1000 ppb concentration of AFB_1. Addition of SB into the contaminated diets corrected the increasing effect of AFB_1 so LDH activity gain decreased and reached to control group.

CONCLUSION

Under the conditions of this study, it was concluded that feeding aflatoxin B_1 at the used levels can impair feed intake, growth performance and efficiency of carcass yield, thigh and breast meat. Using sodium bentonite showed a positive effect to chickens and nearly cleared the negative effect of aflatoxin B_1. It seems that using 0.5-1% sodium bentonite into the diets is the acceptable range to combat the negative effects of aflatoxin B_1 in broiler chickens.

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