Original Article

# Protective effects of milk thistle (*silybum marianum*) seeds and sodium bentonite in ameliorating the toxic effects of aflatoxin b1 in broiler chicks

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## Abstract

**Background:** Aflatoxin-contaminated feed cause mortality, suppression of the immune system, reduced growth rates and losses in feed efficiency. An experiment was conducted to evaluate the ability of different levels of sodium bentonite (SB), Silybummarianum seeds (SMS) and their combination for reducing the adverse effects of Aflatoxin B1 (AFB1) in broiler chicks. **Materials and Methods:** 224 male Ross 308 broiler chicks in a completely randomized design based on factorial experiment (2 × 2 × 2) with 2 levels AFB (0and 500ppb), 2 levels SMS (0 and 0.5%) and 2 levels SB (0 and 0.5%) with 8 treatments, 4 replicates and 7 chickens per each were used from one to 24 days age. **Results:** The results showed that compared with the control group, diets contaminated with $AFB_1$ increased the aminotransferase (AST) and gammaglutamyl transferase (GGT) but decreased serum total protein (TP) and Albumin significantly ($P \leq 0.05$). SB with AFB caused a significant decrease in serum AST and lactate dehydrogenase (LDH) levels and increase Albumin significantly ($P \leq 0.05$). SMS with AFB causes a significant decrease in serum Urea, GGT and LDH levels significantly ($P \leq 0.05$). Broiler chickens fed diets containing SB, SMS and $AFB_1$ their biochemical indices (AST, GGT, Albumin and total protein) were improved significantly compare to basal diet contaminated with aflatoxin ($P \leq 0.05$). **Conclusion:** The results showed that the combination of SB and the SMS were more beneficial as separate application of them for decreased toxic effect of Aflatoxin $B_1$ in diets.

**Keywords:** Aflatoxin $B_1$, Blood parameters, Milk Thistle, sodium bentonite

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## Introduction

Chicken meat is an important source of nutrients for human consumption. Broiler feed is exposed to various contaminants during the process of production, transportation and storage. One of the important injurious agents is mycotoxins as it has hepatotoxic, hepatocarcinogenic, mutagenic and teratogenicity effects in many animal species (IARC, 1987). Mycotoxins are metabolites from moulds. Aflatoxins, a group of closely related biologically active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* [1]. Mycotoxins cause a wide variety of adverse clinical signs, depending on the nature and concentration of toxins in the diets, on the animal species, on their age, and on nutritional and health status at the time of exposure to contaminated feed [2].

The United States Food and Drug Administration (FDA) has set regulatory levels for poultry feeds for 20 ppb AF [3]. Fungi or mould growth in feedstuff is associated with the utilization of nutrients from the host. Consequently, alterations in the nutritional content of the feedstuff are expected. The extent of mould growth determines the degree of depletion in the nutrient content of the feedstuff. The germ of the grain is the main site for *Aspergillus* sp. development, which leads to a greater potential for the synthesis of AFB [4]. Numerous strategies for detoxification or inactivation of mycotoxin in contaminated feedstuff have been used. Most of these techniques are impractical or ineffective [5, 6]. One approach to the detoxification of mycotoxin is the use of nonnutritive absorptive materials in the diet to reduce the absorption of mycotoxins from the gastrointestinal tract [7]. Layered alumino silicates, such as sodium bentonite, have been found effective in counteracting mycotoxins [6, 8]. The effects of *silymarin* or various products made from the seed of the plant *Silybum marianum* containing silymarin have also been tested on farm animals. The hepatoprotective activities were assessed on the basis of selected biochemical blood indicators in chicken broilers that had been exposed to the effects of aflatoxins [1, 9]. It is well established that the health and performance of birds is influenced by the nutrient and metabolites of blood. Therefore with the understanding of relationship between blood biochemical parameters and production characteristics, one can estimate the health and performance of the birds. The purpose of this study was to evaluate the changes of blood biochemical parameters of broiler chickens when different levels of sodium bentonite and *Silybum marianum* seeds as a feed additive in their diet.

# Methods

Two hundred and twenty four day old male Ross 308 broiler chickens obtained from a local hatchery were randomly distributed among eight treatments with four replicate groups per treatment and 7 chickens per replicate. The temperature was maintained at 32°C during the 1st week and then was reduced by 3°C per week. In the one to 24 days continuous lighting was used. The diet was based on corn and soybean meal, containing or exceeding the nutritional requirements recommended by the NRC (1994) but the nutritional requirement of the chicks was based on Ross 308 company for starter (0-10 days) and grower (11-24 days) diets (Table I). The diets of starter phase calculated to contain 23% crude protein (CP) and 2990 kcal of metabolizable energy (ME) per kg of diet and also it contained 21% CP and 3108 kcal of ME per kg of diet for the grower phase and it contained 18.5% CP and 3195 kcal of ME per kg of diet for the finisher phase. This experiment was conducted in a completely randomized design with factorial design (2×2×2). Birds were distributed into the following eight treatments: 1) control 2)diet with (%) 500ppb aflatoxin $B_1$, 3) diet with 500ppb Aflatoxin $B_1$ and 0.5% *Silybummarinum* seeds, 4) diet with 500ppb aflatoxin $B_1$ and 0.5% Sodium Bentonite, 5)diet with 500ppb Aflatoxin $B_1$ and 0.5% *Silybummarinum* seeds and 0.5% Sodium Bentonite, 6)diet with 0.5% *Silybummarinum* seeds, 7) diet with 0.5% Sodium Bentonite, 8) diet with 0.5% *Silybummarinum* seeds and 0.5% Sodium Bentonite. All diets were fed for 4 weeks. The broilers were allowed ad-libitum access to feed and water. The AF production in rice was done according to the method of Shotwell et al [10]. The total AF level was measured on thin layer chromatography (TLC) method as outlined by (AOAC). As a result of the measurement, total AF amount detected in rice flour was 120 ppm. At 24 day of age, two broilers per pen were randomly selected and 2 mL of blood samples were obtained by wing puncture and centrifuged at 3000 rpm for 20 min. The serum was collected and stored at -20°C until analyzed for total proteins, albumin, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT) and lactate dehydrogenase (LDH) were measured on autoanalyzer (Metrolab 2300 plus, Argentina) using commercially available kits. Data were analyzed using the general linear model procedure of SAS (10). Data were subjected to analysis of variance and significant differences ($P \leq 0.05$) observed in means subjected to Tukey's multiple range test.

**Table 1.** Ingredient and chemical composition of the experimental diets starter period (0-10) and grower period (11-24)

| | Starter (0-11 days old) | | | | | | | | Grower (11-24 days old) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
| Corn (%) | 53.74 | 53.74 | 53.42 | 52.68 | 52.36 | 53.42 | 52.68 | 52.36 | 52.93 | 52.93 | 52.61 | 51.87 | 51.55 | 52.61 | 51.87 | 51.55 |
| Soybean (%) | 35.57 | 35.71 | 35.38 | 35.57 | 35.71 | 35.38 | 35.51 | 35.51 | 37.86 | 37.86 | 37.72 | 38.05 | 37.91 | 37.72 | 38.05 | 37.91 |
| Fishmeal (%) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | - | - | - | - | - | - | - | - |
| Fat (%) | 2.61 | 2.61 | 2.57 | 2.97 | 2.93 | 2.57 | 2.97 | 2.93 | 5.19 | 5.19 | 5.15 | 5.55 | 5.51 | 5.15 | 5.55 | 5.51 |
| DcP (%) | 1.03 | 1.03 | 1.03 | 1.03 | 1.04 | 1.03 | 1.03 | 1.04 | 1.59 | 1.59 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 |
| OsterShell (%) | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 |
| Vitamin (%) | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral (%) | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt (%) | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| DL- methionine (%) | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| L- lysine (%) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SB (%) | - | - | - | 0.5 | 0.5 | - | 0.5 | 0.5 | - | - | - | 0.5 | 0.5 | - | 0.5 | 0.5 |
| SMS (%) | - | - | 0.5 | - | 0.5 | 0.5 | - | 0.5 | - | - | 0.5 | - | 0.5 | 0.5 | - | 0.5 |
| $AfB_1$ (ppb) | - | 500 | 500 | 500 | 500 | - | - | - | - | 500 | 500 | 500 | 500 | - | - | - |
| Nutrient (%) | | | | | | | | | | | | | | | | |
| Energy(kcal/kgr) | 2990 | 2990 | 2990 | 2990 | 2990 | 2990 | 2990 | 2990 | 3108 | 3108 | 3108 | 3108 | 3108 | 3108 | 3108 | 3108 |
| Protein (%) | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| Lysine (%) | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.20 | 1.20 | 1.19 | 1.20 | 1.20 | 1.19 | 1.20 | 1.20 |
| Met + Cys (%) | 0.74 | 0.74 | 0.73 | 0.74 | 0.73 | 0.73 | 0.74 | 0.73 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| Tryptophan (%) | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.28 | 0.28 | 0.27 | 0.28 | 0.27 | 0.27 | 0.28 | 0.27 |
| Calcium (%) | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 |
| Phosphorus (%) | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 |
| Fiber (%) | 3.87 | 3.87 | 3.98 | 3.86 | 3.97 | 3.98 | 3.86 | 3.97 | 3.97 | 3.97 | 4.07 | 3.96 | 4.06 | 4.07 | 3.96 | 4.06 |

[1.] Provided per kilogram of diet: vitamin A, 1,100 IU (as retinyl acetate); vitamin D3, 2,200 IU (as cholecalciferol); vitamin E, 30 mg (as α-tocopheryl acetate); vitamin K3, .5 mg (as menadione sodium bisulfite); vitamin B12, .02 mg; vitamin niacin, 60 mg; folic acid, 0.6 mg; biotin, 0.15 mg; choline chloride, 788 mg; B1, 2 mg (thiamine); B6, 5 mg (as pridoxamine).

## Results

The effect of experimental treatmets on blood parameters in table 2 show that incorporation of 500ppb aflatoxin with basal diet significantly decreased the amount of albumin (P≤0.05). 0.5% Sodium bentonite in the diet contaminated with aflatoxin significantly increased serum albumin level (P≤0.05). The combination of 0.5 percent sodium bentonite and 0.5 percent of Silybum marianum seeds in the diet contaminated with aflatoxins significantly increased level of albumin compared with the control group (P≤0.05). In comparison with the control which had combination of sodium bentonite 0.5% and 0.5 percent of Silybum marianum seeds to this diet resulted in a significant increase in serum total protein (P≤0.05). The result in the main effect of creatinine showed that 500ppb AFB significantly increased creatinine serum level compare to zero level of AFB (P≤0.05). Statistical analysis of the main effect showed that 0.5 percent SMS significantly decreased urea level compared to zero level of SMS (P≤0.05). In table 3, three way interaction between treatments showed contaminated control group (5 treatment) increased significantly AST serum levels compared to control group (P≤0.05). Combine 0.5 percent SB in contaminated diets with AFB decreased significantly AST serum level (P≤0.05). Separately combine 0.5 percent SMS in contaminated diets with AFB decreased significantly AST serum level (P≤0.05). Combination of 0.5% SB and 0.5% SMS in diets containing AFB decreased significantly AST serum level (P≤0.05). Also diet containing aflotoxin significantly increased GGT serum level (P≤0.05). Two way interaction analyses in table 3 showed that interaction between 500ppb level AFB and zero level SMS caused significant increase in ALT serum level and compared with this. Interaction between 500ppb level AFB and 0.5 percent level SMS caused significant decrease in ALT serum level (P≤0.05) 500ppb level. Interaction between 500ppb level AFB and 0.5 percent level SMS caused significant decrease LDH serum level (P≤0.05). Interaction between 500ppb level AFB and zero percent level SB caused significant increase in LDH serum level (P≤0.05). The effects of different diets on blood parameters are shown in table 1.

## Discussion

AFB has been shown to cause inhibition of protein synthesis [11]. Albumin and total protein levels in the serum proved to be sensitive indicators of aflatoxicosis in broilers. Use of absorbents in broiler diet will cause the subsequent absorption of toxin in their digestive tract and this prevents the reduction of blood serum albumin, globulin and protein in the case of using contaminating diet [12]. Our results are consistent with the findings reported have shown that some serum biochemical changes could be ameliorated by bentonite administration to the diet at doses of 5 mg/kg in broiler chickens given 2.5 mg AF/kg diet [13]. However, in our case, the biochemical parameters for broilers fed diets containing SB + AF did not completely return to normal values, showing an inhibition of protein synthesis. These results are parallel with the findings reported [11], who observed a decrease in these parameters and an inhibition in protein synthesis during the aflatoxicosis in poultry.

Silybum marianum and bentonite sodium supplementation to a contaminated diet significantly improved albumin values, probably as a result of effective adsorption in the gut to reduce the amount of AF absorption by the body and hepatoprotective effect in liver. The increased serum GGT, AST, and LDH activities observed by feeding naturally contaminated diets in the present study could be due to hepatic degeneration and subsequent leakage of enzymes into circulation. Similar increases in the activities of GGT as observed in the present trial have been reported during aflatoxicosis in broiler breeder hens [14]. These observations are consistent with the studies in which an increase in GGT activity in the serum was reported [3].

## Conclusion

Dietary inclusion of sodium bentonite had not any adverse effects on serum biochemical characteristics and it can also improve the values of serum total proteins and albumin in broiler chickens also results of the present study showed that consumption of level of SMS from 0.5 % in poultry diets can reduce the toxicity of AFB1 in broiler chickens. But results showed that the combination of

**Table 2:** Effect of different treatment on blood parameters

| | | | | Albumin | TP | Creatinine | Urea |
|---|---|---|---|---|---|---|---|
| Main effects | Aflatoxin (ppm) | | 0 | 1.43[a] | 2.50 | 0.25[b] | 3.5 |
| | | | 500 | 1.35[b] | 2.54 | 0.30[a] | 3.5 |
| | p-value | | | 0.00 | 0.63 | 0.00 | 1 |
| | Silybum (%) | | 0 | 1.36[b] | 2.33[b] | 0.28 | 3.75[a] |
| | | | 0.5 | 1.42[b] | 2.71[a] | 0.27 | 3.25[b] |
| | p-value | | | 0.03 | 0.00 | 0.08 | 0.04 |
| | Bentonite (%) | | 0 | 1.29[b] | 2.35[b] | 0.28 | 3.5 |
| | | | 0.5 | 1.49[a] | 2.70[a] | 0.28 | 3.5 |
| | p-value | | | 0.00 | 0.00 | 0.94 | 1 |
| | SEM | | | 0.01 | 0.05 | 0.00 | 0.16 |
| Dual interaction effects | Aflatoxin * silybum | | | | | | |
| | 0 | 0 | | 1.45[a] | 2.45[bc] | 0.26 | 3.75 |
| | 0 | 0.5 | | 1.41[a] | 2.56[b] | 0.24 | 3.25 |
| | 0 | 500 | | 1.27[b] | 2.21[c] | 0.31 | 3.75 |
| | 0.5 | 500 | | 1.43 | 2.87[a] | 0.29 | 3.75 |
| | p-value | | | 0.00 | 0.00 | 0.94 | 1 |
| | Aflatoxin * Ben | | | | | | |
| | 0 | 0 | | 1.42[b] | 2.43[bc] | 0.25 | 3.75 |
| | 0 | 0.5 | | 1.45[ab] | 2.57[ab] | 0.25 | 3.25 |
| | 0 | 500 | | 1.17[c] | 2.26[c] | 0.30 | 3.25 |
| | 0.5 | 500 | | 1.52[a] | 2.82[a] | 0.30 | 3.75 |
| | p-value | | | 0.00 | 0.01 | 0.83 | 0.05 |
| | Silybum * Ben | | | | | | |
| | 0 | 0 | | 1.29 | 2.21 | 0.29 | 3.62 |
| | 0 | 0.5 | | 1.43 | 2.45 | 0.28 | 3.87 |
| | 0 | 500 | | 1.30 | 2.48 | 0.27 | 3.37 |
| | 0.5 | 500 | | 1.54 | 2.95 | 0.27 | 3.12 |
| | p-value | | | 0.06 | 0.16 | 0.43 | 0.30 |
| | SEM | | | 0.02 | 0.07 | 0.00 | 0.23 |
| Treatments | AFB * silybum * Ben | | | | | | |
| | 0 | 0 | 0 | 1.59[a] | 2.35[bc] | 0.27 | 4.25 |
| | 0 | 0 | 0.5 | 1.40[bc] | 2.55[bc] | 0.25 | 3.25 |
| | 0 | 0.5 | 0 | 1.34[bc] | 2.52[bc] | 0.24 | 3.25 |
| | 0 | 0.5 | 0.5 | 1.49[ab] | 2.60[b] | 0.25 | 3.25 |
| | 500 | 0 | 0 | 1.08[d] | 2.07[c] | 0.31 | 3 |
| | 500 | 0 | 0.5 | 1.46[ab] | 2.35[bc] | 0.31 | 4.5 |
| | 500 | 0.5 | 0 | 1.26[cd] | 2.45[bc] | 0.30 | 3.5 |
| | 0.5 | 0.5 | 500 | 1.50[ab] | 3.30[a] | 0.29 | 3 |
| | p-value | | | 0.01 | 0.03 | 0.14 | 0.05 |
| | SEM | | | 0.03 | 0.11 | 0.01 | 0.33 |

Means within a column that do not have a common superscript are significantly different (P<0.05).
SEM= Standard error of means. AFB= Aflatoxin $B_1$. Ben= Bentonite

SB and the SMS are more beneficial as separate application of them for decreased toxic effect of Aflatoxin $B_1$ in diets.

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**Table 3:** Effect of different treatment on blood parameters

| | | | | AST | ALT | GGT | LDH |
|---|---|---|---|---|---|---|---|
| Main effects | Aflatoxin (ppm) | 0 | | 179.31$^{b}$ | 6.68$^{b}$ | 21.56$^{b}$ | 687.69$^{b}$ |
| | | 500 | | 208.90$^{a}$ | 8.87$^{a}$ | 25.65$^{a}$ | 943.56$^{a}$ |
| | p-value | | | 0.00 | 0.00 | 0.00 | 0.00 |
| | Silybum (%) | 0 | | 196.18 | 8.25$^{a}$ | 24.45$^{a}$ | 857.06$^{a}$ |
| | | 0.5 | | 192.03 | 7.31$^{b}$ | 22.76$^{b}$ | 774.19$^{b}$ |
| | p-value | | | 0.25 | 0.03 | 0.00 | 0.00 |
| | Bentonite (%) | 0 | | 206.58$^{a}$ | 8 | 22.87$^{b}$ | 840.19$^{a}$ |
| | | 0.5 | | 181.63$^{b}$ | 7.56 | 24.34$^{a}$ | 791.06$^{b}$ |
| | p-value | | | 0.00 | 0.31 | 0.01 | 0.01 |
| | SEM | | | 2.49 | 0.29 | 0.38 | 12.61 |
| Dual interaction effects | Aflatoxin * silybum | | | | | | |
| | 0 | 0 | | 178.50 | 6.62$^{b}$ | 20.81$^{c}$ | 687.25$^{c}$ |
| | 0 | 0.5 | | 180.13 | 6.75$^{b}$ | 22.30$^{bc}$ | 687.75$^{c}$ |
| | 0 | 500 | | 213.86 | 9.87$^{a}$ | 28.08$^{a}$ | 1026.50$^{a}$ |
| | 0.5 | 500 | | 203.93 | 8.87$^{b}$ | 23.22$^{b}$ | 860.62$^{b}$ |
| | p-value | | | 0.11 | 0.01 | 0.00 | 0.00 |
| | Aflatoxin * ben | | | | | | |
| | 0 | 0 | | 175.58$^{b}$ | 6.37$^{c}$ | 21.17 | 662.50$^{c}$ |
| | 0 | 0.5 | | 183.03$^{b}$ | 7$^{bc}$ | 21.95 | 712.87$^{c}$ |
| | 0 | 500 | | 237.58$^{a}$ | 9.62$^{a}$ | 24.57 | 1017.87$^{a}$ |
| | 0.5 | 500 | | 180.21$^{b}$ | 8.12$^{ab}$ | 26.73 | 869.25$^{b}$ |
| | p-value | | | 0.00 | 0.01 | 0.21 | 0.00 |
| | Silybum * ben | | | | | | |
| | 0 | 0 | | 215.72$^{a}$ | 8.37 | 24.73$^{a}$ | 850.62$^{a}$ |
| | 0 | 0.5 | | 176.63$^{c}$ | 8.12 | 24.17$^{a}$ | 863.50$^{a}$ |
| | 0 | 500 | | 197.45$^{b}$ | 7.62 | 21.01$^{b}$ | 829.75$^{a}$ |
| | 0.5 | 500 | | 186.62$^{bc}$ | 7 | 24.51$^{a}$ | 718.62$^{b}$ |
| | p-value | | | 0.00 | 0.66 | 0.00 | 0.00 |
| | SEM | | | 3.52 | 0.42 | 0.55 | 17.83 |
| Treatments | AFB * silybum * Ben | | | | | | |
| | 0 | 0 | 0 | 177.12$^{c}$ | 6.25 | 19.82$^{b}$ | 630 |
| | 0 | 0 | 0.5 | 179.87$^{c}$ | 7 | 21.82$^{b}$ | 745.25 |
| | 0 | 0.5 | 0 | 174.5$^{c}$ | 6.5 | 22.52$^{b}$ | 695 |
| | 0 | 0.5 | 0.5 | 186.22$^{c}$ | 7 | 22.07$^{b}$ | 680.50 |
| | 500 | 0 | 0 | 254.32$^{a}$ | 10.50 | 29.65$^{a}$ | 1071.25 |
| | 500 | 0 | 0.5 | 173.40$^{c}$ | 9.25 | 26.52$^{a}$ | 981.75 |
| | 500 | 0.5 | 0 | 220.85$^{b}$ | 8.75 | 19.50$^{b}$ | 964.50 |
| | 0.5 | 0.5 | 500 | 187.02$^{c}$ | 7 | 26.95$^{a}$ | 756.75 |
| | p-value | | | 0.01 | 0.88 | 0.00 | 0.87 |
| | SEM | | | 4.98 | 0.59 | 0.77 | 25.22 |

Means within a column that do not have a common superscript are significantly different ($P$<0.05).
SEM= Standard error of means. AFB= Aflatoxin $B_1$. Ben= Bentonite

sampling process.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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