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Effects of Selenium and Zinc in Aflatoxin B1 Poisoning in Broiler

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

To test the effect of selenium & zinc in aflatoxin B1 poisoning in broiler, day-old broiler chicks (n=80) were divided into 5 treatment groups with 16 birds each, (T0-control; T1-50ppb AFB1 T2-100ppb AFB1, T3-200ppb AFB1, T4-400ppb AFB1), after treating 3 weeks, 8 birds were sacrificed & rest of the birds continued with AFB1 & started a new treatment with Zn & Se supplements at the dose rate Zn (40, 60, 80 mg/kgbwt) and Se (30, 40, 50 µg/kgbwt) respectively in T2, T3 & T4 from 3rd week to 5th week. Experiment was conducted from day 1 to 35 days of age. The broilers were weighed, bled, killed by cervical dislocation .Body weights were significantly decreased (643.66±3.17) by 400ppb aflatoxin B1 ($P < 0.05$) at 21 days compare to control group T0. Higher concentration aflatoxin B1 (400ppb) in T4 group significantly decreased body weight from T1 (50ppb). Aflatoxin B1 induced a significant increase biochemical parameters such as serum alanine transaminase (ALT) & aspartate transaminase (AST).Significantly elevated level ($P < 0.05$) of ALT & AST were observed in T4 (58.2±0.7 & 1185±17) respectively. In case of hematological parameters, the study revealed that it also reduced the level of total erythrocyte count (TLC), total leucocyte count (TLC), hemoglobin, lymphocyte, monocyte & basophil, however it accelerated neutrophil & eosinophil level. Aflatoxin B1 raised weight of liver, spleen & diminished weight of pancreas. Higher concentration extended mortality rate upto 15% in T4 group at 3 weeks. After treating with Zn& Se, growth performances had been improved gradually, it depressed these negative effects of AFB1 in broiler. These effects of AFB1 were ameliorated by supplementation of Zn & Se. It was concluded that aflatoxin contamination (50, 100, 200, 400 ppb) in broiler diet

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impaired the performance in terms of body weight gain, biochemical, hematological parameter, relative organ weights & livability percentage. Supplementation of zinc & Se in the aflatoxin contaminated diet improved the adverse effects of aflatoxicosis on performance of the broiler chickens. Supplementation. In conclusion, the results demonstrated that mineral treatment (Zn & Se) might be used for correction of aflatoxin B1 in broiler.

Keywords: Aflatoxin B1; aflatoxicosis; alanine transaminase alanine aminotransferase; lymphocytopenia; monocytopenia total erythrocyte count total leucocyte count; parts per billion.

1. INTRODUCTION

"According to an estimate by the food and agricultural organization (FAO), 25% of the worlds food crops are affected by mycotoxins and the rate of mycotoxin contamination is likely to increase in line with the trend seen in preceding years" [1]. "A worldwide mycotoxin survey in 2013 revealed that 81% of around 3000 grain and feed samples analyzed had at least one mycotoxin which was higher than the 10 year average (from 2004 to2013) of 76% in a total of 25,944 samples. The most notorious mycotoxins are aflatoxins, which often result in low performance in poultry and decreased quality of egg and meat production and then cause significant economic losses" [2].

"Contamination of food or feedstuffs and their consumption can result in mycotoxicosis. In these mycotoxins, aflatoxins are the mostly seen and aflatoxin B1 is the most harmful one" [3,4]. "Synthesis of aflatoxins in feeds is increased at temperatures above 27◦C (80 F), humidity levels greater than 62%, and moisture levels in the feed above 14%" [5]. "Aflatoxicosis causes several defects in organs and tissues, decrease in growth rate, increase in death rate, immunosuppression, anemia, and increase in coagulation time and deteriorates lipid, carbohydrate and protein metabolism" [6,7]. "Significant changes in serum biochemical and hematological parameters are seen in aflatoxicosis cases, and these can assist in the diagnosis of toxications" [8,9]. "Changes in the composition of poultry diets (dietary modifications) alleviate the adverse effects of aflatoxin in the poultry. Dietary fortification with certain vitamins, fat [10], protein [11], fatty acids [12] and trace mineral (Zn, Cr, Se) [13] reduce the effect of aflatoxin on the performance of poultry". Zinc participates in antioxidant defense mechanism as cofactor of super-oxide dismutase. Keeping these points, the present study was carried out to evaluate the adverse effect of AFB1 in broiler growth performance, hematological & biochemical parameters & also investigate the effect of Zn and Se in aflatoxin B1 poisoning in broiler.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at the SAU Poultry Farm, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

2.2 Management of the Chickens

All the management procedures were followed as possible as per poultry science standard. The experimental poultry house was thoroughly cleaned and disinfected. It was demarcated into sixteen pens by using chicken wire and wooden poles. The birds were kept on the litter floor with 16 birds per pen. They had a free access to feed and water. Fresh feed and clean drinking water had to be supplied every day. Brooding facilities had ready in the house.

2.3 Experimental Diets and Treatments

A total of 80, day-old broiler chicks were purchased from Kazi hatchery. The initial body weights of assigned chickens were taken with digital weight machine and are recorded in every week. The experiment was conducted according to the completely randomized design. Birds were divided into 5 equal groups (T0, T1, T2, T3 & T4) with 16 birds. Group T0 was considered as untreated control feeded with commercial feed only, group T1, T2, T3 & T4 supplemented with AFB1 at the dose rate of 50ppb, 100ppb, 200ppb, and 400ppb respectively upto 5 weeks. After 3weeks 8 birds slaughtered for pathological and biochemical test for observing AFB1 effect & rest of the birds continued with AFB1 & started a new treatment with Zn & Se supplements at the dose rate Zn (40, 60, 80 mg/kgbwt) and Se (30, 40, 50 µg/kgbwt) respectively in T2, T3 & T4

from 3rd week to 5th week. T1 group continued with same treatment AFB1. Finally rest of birds were Sacrificed at 5 week of age. Powered AflatoxinB1, Zn & Se were incorporated in the normal feed so as to above mention level in the feed.

2.3.1 Source of ration

Readymade commercial broiler ration from renowned feed industry was used.

2.4 Study Parameters

The parameters to be recorded: weekly live weight, Livability percentage, Hematological, biochemical parameter, organ weight after treating AFB1, effect of Se and Zn in AFB1 treated birds.

2.5 Hematology

Blood were collected from wing vein in non-sacrificing bird. In Prior to sacrifice, blood was collected in heparinized vials by cardiac puncture for hematological & biochemical studies. The hemoglobin (Hb, Sahli's acid haematin methods), total erythrocyte count (TEC, Neubaur's chamber), total leucocyte count (TLC, Neubaur's chamber) & differential leucocyte count (DLC, Wright's stain) estimations were carried out. The red blood cell (RBC) and white blood cell (WBC) were determined by haematocytometer; haemoglobin amounts were determined by commercial kit (Biosystem), and differential leukocyte counts were determined in blood smears [14].

2.5.1 Serum biochemistry

Blood samples were also collected at each interval in non-heparinized tubes. The sera were separated and analyzed for the alanine aminotransferase (ALT), alkaline phosphatase (ALP) by using Automatic biochemical analyzer. All biochemical estimations carried out within 24 hours of collections.

2.6 Statistical Data Analysis

Statistical differences among the groups were tested by analysis of variance (ANOVA) which is followed by Duncan's test using SPSS for windows version 16.0 [15]. Significant were considered as $P < 0.05$ [15].

3. RESULTS AND DISCUSSION

3.1 Effect on Body Weight

Table 1 shows the effect of the dietary treatments AFB1 & Zn & Se on regular body performance during the experiment period. The mean body weights of broilers in different groups showed that the average weight gain (g) increased in birds fed with normal diet from day 0 up to day 35 (227.33 ± 1.76^{b}, 555.33 ± 5.17^{a}, 1023 ± 12.12^{b}, 1611.6 ± 56.74^{b}, 2097 ± 55.15^{ab}) but significant decreases of body weight after treating AFB1 in each treatment group in using different concentration. Higher concentration of AFB1 is highly affecting in growth performance (215 ± 2.88^{c}, 290 ± 5.77^{a}, 643.66 ± 3.17^{c}, 901.66 ± 14.24^{c}, 1423.3 ± 38.44^{ac}) in broiler up to 3weeks compare to control group T0. After 3 weeks, T2, T3 & T4 groups are treated with Se + Zn in different concentration, after treating with Zn & Se, it gradually improved the growth performances compare to Zn & Se untreated group T1 up to 5 weeks. This report was in accordance with the findings of [16] who concluded in their review that each mg of AFB1/kg diet would decrease the growth performance of broilers by 5% .For instance; [17] noted 21% decrease in final body weight at 35 days age in broilers fed on 0.3 mg AFB1/kg diet. Contrary to this, [18] noted only 10% reduction in weight gain of broilers at 28 days of exposure to 0.8 mg AFB1/kg diet. A significant improvement in RBG (relative body gain) in the Zn and Se + Zn dietary enrichment groups was observed [19]. This result strongly agreed with that Zn & Se are an important determinant of growth performance in broiler. However, Se is also useful for the improvement of immune responses during salmonellosis, with or without aflatoxicosis. In the present study, addition of zinc & selenium resulted in significant improvement in body weight during aflatoxicosis. This finding is in agreement with these words [20] where zinc fortification (60 mg/kg) in diet resulted in significant improvement infeed efficiency of chicks exposed to aflatoxicosis.

Table 1. Body weight (gram) values (Mean+SE) in experimental chicks of different groups at various intervals

Parameters	Intervals	T(0)	T1(50)	T2(100)	T3 (200)	T4 (400)
Body Weight (g)	1st weeks	227.33±1.76^{b}	224.33±3.28^{c}	214± 4.72^{ab}	218±1.73^{b}	215±2.88 ^{c}
	2nd weeks	555.33±5.17^{a}	513.33±8.81^{a}	337.66±6.22^{c}	300±5.77 ^{c}	290±5.77 ^{a}
	3rd weeks	1023±12.12 ^{b}	962.33± 31.39^{c}	692±4.16^{c}	691±6.02 ^{a}	643.66±3.17^{c}
	4th weeks	1611.66±56.74^{b}	1386.66±116.3^{ac}	943.33±29.62^{b}	935.66±33.19^{bc}	901.66±14.24^{c}
	5th weeks	2097±55.15^{ab}	1766.66±120.1^{b}	1393.33±54.56^{a}	1440±30.55^{b}	1423.33±38.44^{ac}

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

3.2 Effect on Biochemical Parameter

Table 2 showed AFB1 effect on biochemical parameter such as serum ALT & ALP. Significantly elevated levels of alanine transaminase (ALT) & aspartate transaminase (AST) levels observed in the present investigation might be due to damage to the hepatocyte and consequent release of enzyme in the aflatoxicated (II and III) in broilers, which have also been reported earlier in broiler birds during experimental aflatoxicosis [21]. In chicken, the activity of serum or plasma enzymes like the sorbitol dehydrogenase, glutamic dehydrogenase, lactate dehydrogenase, alkaline phosphatase, acid phosphatase, aspartate aminotransferase and alanine aminotransferase were reported to be increased in aflatoxicated chickens [22], it support this work also. After 3 weeks T2, T3 & T4 groups was treated with Zn & Se in different concentration, after 2weeks treatment T2, T3 & T4 groups showed significant decrease of ALT & ALP level in serum gradually was coming to normal compare to T0 groups. That's mean Zn & Se also contributes in improvement of health & liver damage.

3.3 Effect on Hematological Parameter

The result showed that decreased level of TEC, TLC & Hemoglobin in AFB1 treated group compare to control group T0 up to 3 weeks, After 3 weeks T2, T3 & T4 groups treated with Zn & Se after that, TEC, TLC & Hemoglobin level increase gradually with treatment concentration compare with only AFB1 treated group T1. Reduction in hemoglobin concentration during in aflatoxin b1 treated birds observed in the study was in accordance with the reports of earlier workers [23]. Reduction in total leucocytes count in aflatoxicosis was almost in agreement with several previous reports of [24,25] who observed leukocytopenia in broilers at relatively lower level of aflatoxicosis (100 ppb & 150 ppb). However, still yet, there has no accurate report of effect of Zn & Se in blood parameter of aflatoxin B1 affected broiler chicken.

Table 4 result showed that neutrophil & eosinophil amount increased & lymphocyte, monocyte & basophil amount were decreased compare to control group T0 respectively with concentration up to 3 weeks, After that broilers were treated with Zn & Se in different concentration at T2, T3, T4 groups, data showed that neutrophil & eosinophil level diminished gradually & lymphocyte, monocyte & basophil value up winded compare to T1 group. The researchers reported that aflatoxicosis caused the lymphocytopenia and monocytopenia but increased the WBC and percentage of heterophil counts [26-29]. This increase in WBC and percentage of heterophil counts suggest that the toxin elicited an inflammatory response. However, the decreases in the other percentage of leukocyte types may be related with relative reduction [30,31].

Table 2. Biochemical parameter (U/L) values (Mean+SE) in experimental chicks of different groups at various intervals

Parameters	Intervals	T0	T1	T2	T3	T4
Serum ALT	1st week	16.1^{b}±3.1	15.1^{c}±1.6	16.1^{a}± 0.8	18.2^{bc}±2.4	19.4^{c}±4.0
	2nd week	16.3^{a}±2.7	21.7^{c}±0.7	24.5^{c}±0.6	27.0^{a}±0.3	29.0^{b}±0.4
	3rd week	16.0^{a}±3.2	30.2^{a}±0.5	38.2^{b}±0.8	45^{b}±0.8	58.2^{a}±0.7
	4th week	15.6^{c}±4.1	40.8^{ab}±0.7	28.3^{a}±1.0	30.0^{c}±0.6	40.2^{bc}±0.9
	5th week	15.4^{ab}±3.9	63.3^{ca}±1.6	20.1^{c}±0.7	23.4^{a}±0.9	30.0^{c}±0.8
Serum ALP	1st week	632.8^{b}±7	641.5^{c}±4.9	638.9^{b}±4.6	645.6^{c}±10	637.4^{a}±11
	2nd week	640.2^{c}±15	662^{a}±4.0	669^{a}±3.9	682^{a}±9.4	745^{a}±4.3
	3rd week	642.3^{a}±12	690^{a}±3.9	910^{bc}±4.2	990^{ac}±10.2	1185^{ab}±17
	4th week	644.5^{ca}±14	734^{a}±5.02	660^{bc}±2.4	690^{bc}±11.3	850^{c}±12
	5th week	645.6^{c}±14.5	792^{bc}±8.4	640^{c}±3.5	645^{c}±12	690^{ca}±8.4

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

Table 3. Hematological values (Mean+SE) in experimental chicks of different groups at various intervals

Parameters	Intervals	T0	T1	T2	T3	T4
TEC(X106cumm)	3rd week	3.8^{b}±0.14	3.5^{c}±0.2	3.02^{b}±0.8	2.75^{b}c±0.9	2.43^{c}±0.36
	5th week	4.0^{b}±0.15	3.3^{b}±0.16	3.4^{c}±0.14	3.67^{c}±0.15	3.9^{a}±0.14
TLC (X103cumm)	3rd week	23.02^{c}±1.4	20.9^{a}±1.08	18.75^{c}±2.3	16.00^{a}±1.5	13.45^{c}±0.85
	5th week	25.02^{c}±1.32	20.07^{a}±1.23	21.87^{c}±1.19	22.50^{b}±1.17	24.75^{bc}±1.29
Hemoglobin (g/dl)	3rd week	11.87^{bc}±0.48	9.0^{b}±0.38	7.50^{a}±0.28	6.85^{b}±0.42	5.9^{a}±0.25
	5th week	12.18^{b}±0.43	8.76^{a}±0.38	9.20^{c}±0.37	9.6^{a}±0.41	11.48^{a}±0.34

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

Table 4. Differential leucocytes count (%) (Mean+SE) in experimental chicks of different groups at various intervals

Parameters	Intervals	T0	T1	T2	T3	T4
Neutrophil	3rd week	33.5^{b}±1.48	38.0^{ca}±2.18	42.4^{ba}± 1.9	46^{a}±1.7	50.50^{b}±1.50
	5th week	34.1^{b}±1.42	39.76^{a}±2.10	38.50^{a}±1.50	37.50^{c}±1.56	35.7^{b}±1.46
Lymphocyte	3rd week	62.53^{c}±1.10	57.87^{a}±2.04	53.50^{a}±1.87	49.54^{bc}±1.80	45.61^{ab}±1.78
	5th week	61.54^{cb}±1.2	56.10^{a}±1.87	57.56^{c}±1.65	58.55^{c}±1.54	60.34^{a}±1.48
Monocyte	3rd week	2.12^{b}±0.40	1.92^{c}±0.25	1.72^{a}±0.23	1.54^{c}±0.22	1.1^{a}±0.19
	5th week	2.10^{a}±0.30	1.87^{a}±0.21	1.90^{ac}±0.22	1.95^{b}±0.19	2.0^{a}±0.18
Eosinophil	3rd week	1.35^{c}±0.35	1.8^{c}±0.28	2.0^{a}±0.24	2.6^{b}±0.30	3.0^{c}±0.23
	5th week	1.45^{b}±0.27	1.85^{c}±0.25	1.6^{b}±0.23	1.54^{ab}±0.21	1.48^{c}±0.20
Basophil	3rd week	0.50^{b}±0.17	0.41^{a}±0.17	0.38^{c}±0.16	0.32^{c}±0.11	0.29^{a}±0.12
	5th week	0.50^{c}±0.18	0.42^{a}±0.18	0.44^{b}±0.19	0.46^{b}±0.17	0.48^{c}±0.16

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

Table 5. Relative weights of organs (% of live weight) fed different dietary treatment

Parameters	Intervals	T0	T1	T2	T3	T4
Liver		2.4^{b}±0.09	2.80^{c}±0.07	2.90^{ab}±0.06	3.20^{c}±0.04	3.5^{a}±0.05
Spleen	3rd week	0.25^{c}±0.01	0.27^{a}±0.02	0.30^{a}±0.01	0.34^{bc}±0.02	0.37^{b}±0.03
Bursa		0.18^{ab}±0.02	0.16^{c}±0.03	0.13^{b}±0.04	0.11^{ac}±0.01	0.9^{a}±0.01
Liver		2.38^{c}±0.04	3.2^{a}±0.03	2.7^{a}±0.07	2.50^{c}±0.09	2.37^{b}±008
Spleen	5th week	0.24^{c}±0.02	0.30^{b}±0.01	0.27^{a}±0.03	0.26^{b}±0.02	0.25^{c}±0.01
Bursa		0.19^{b}±0.02	0.10^{ac}±0.01	0.15^{c}±0.04	0.16^{a}±0.02	0.17^{a}±0.03

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

Table 6. Livability percentage ((Mean+SE) as influenced by different dietary treatment

Treatment groups	1st week	2nd week	3rd week	4th week	5th week
T0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
T1	100.0±0.0	100.0±0.0	97.5±2.5	97.5±2.5	95.0±3.06
T2	100.0±0.0	97.50±2.5	95.0±3.06	95.0±3.06	95.0±3.06
T3	100.0±0.0	95±3.06	90.00±2.50	90.00±2.50	90.00±2.50
T4	100.0±0.0	92.50±3.06	85.00±2.50	85.00±2.50	85.00±2.50

**T0-control, T1-AFB1 50ppb, T2- AFB1 100ppb, T3- AFB1 200ppb, AFB1 400ppb upto 5 weeks & after 3 week treatment started in T2, T3 & T4 with Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) concentration respectively. Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.05)*

3.4 Effect on Organ Weight

In the present study, contamination of aflatoxin at 400 ppb level in the diet of broiler chickens resulted in significant (P<0.05) increase in the relative weight of liver (Table 5). Similar observations were also reported a significant increase in the relative weight of liver due to 300 ppb of aflatoxin contamination in the diet of broilers [32]. Significant increase in relative spleen weight due to dietary aflatoxin content ranging from 3.5 to 5 ppm was also reported by earlier researchers [33]. This study revealed that supplementation of zinc & selenium at Zn (40, 60, 80 mg/kgbwt) & Se (30, 40, 50 µg/kgbwt) to the aflatoxin contaminated diet reversed the effect of aflatoxin on relative weight of spleen. The relative weight of bursa of Fabricius in control group (T0) was higher (P<0.05) than that of aflatoxin alone fed group (T1, T2, T3, T4) upto 3 weeks. A significant (P<0.05) decrease in the weight of bursa was reported at 400 ppb level of dietary aflatoxin. These results corroborated well with earlier reports such as a significant decrease in the relative bursa weight was observed at 300 ppb level of aflatoxin in the diet [34,35]. Result indicated that, supplementation of zinc & selenium to the aflatoxin contaminated diet ameliorated the ill effect of aflatoxin on relative weight of bursa of Fabricius.

3.5 Effect on Livability Percentage

At sixth week of age, the livability percentage in control group was 100 which numerically reduced to 85.00 in T4 up to 3 weeks. Research study reported that an increase in mortality due to 300 ppb level of aflatoxin in the diet of broilers [36,37]. The livability percentage in groups T2, T3 and T4 was stiffed after treating with Zn & Se that indicate that Zn & Se contribute in improvement of livability percentage in broiler chickens.

4. CONCLUSION

It was concluded that 50, 100, 200 & 400 ppb aflatoxin in broiler diet impaired the performance in terms of body weight gain & relative organ weights, biochemical & hematological parameter. It also indicates that supplementation of Zn (40, 60, 80 mg/kgbwt) and Se (30, 40, 50 µg/kgbwt) respectively in the aflatoxin contaminated diet improved the adverse effects of aflatoxicosis on performance of the broiler chickens.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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