

## EFFECT OF BENTONITE, SEPIOLITE, *Coriobacteriaceae* AND FUMONISIN ESTERASE ON GROWTH RATE, BLOOD PARAMETERS, CHICKEN MEAT QUALITY, AND AFLATOXIN RESIDUES

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**Abstract:** Aflatoxin contaminates crops like corn and soya that comprise the main components of chicken ration. Aflatoxin led to deleterious effect on chicken and human health consume chicken meat, so two commercial antimycotoxins were evaluated in current study. Ninety Avian 48 broiler chickens were divided into three groups (30 birds each). The first group received a naturally contaminated ration contained (36±0.21 µg/kg total aflatoxin). The second group (T1) was fed the contaminated ration supplemented with (Bentonite 55.6 % -Sepiolite 44.4 %). The third group (T2) was given a contaminated ration containing (Bentonite 65 % -*Coriobacteriaceae* 0.85x10<sup>11</sup> CFU –fumonisin esterase 7500 IU). Aflatoxin significantly reduced (p < 0.05) body weight gain 1403 ± 57.4g in the control group compared with T1 (1748±72.94g) or T2 (1681±86.05g) at 35 days of age. The Hb concentration and RBC count were (9.33 ± 0.30 and 2.82 ± 0.11 x10<sup>6</sup>/µl) in T1 and (10.48 ± 0.25 and 3.29 ± 0.07 x10<sup>6</sup>/µl) in T2, but significantly lower in the control group (8.08 ± 0.11 and 2.22 ± 0.08 x10<sup>6</sup>/µl). The water holding capacity (WHC) in T1 and T2 was 61.23 ± 1.34% and 62.12 ± 1.33%, respectively. In control it was 56.64 ± 1.23%. The pH value was 6.22 ± 0.14, 6.14 ± 0.12, and 6.11 ± 0.13 in the control, T1 and T2, respectively. Thiobarbituric acid (TBA) and Total volatile basic nitrogen (TVB-N) were significantly higher in control group (0.45 ± 0.05 mg/ kg and 11.2 ± 0.35 mg/100g) than T1 (0.37 ± 0.04 mg/kg and 9.6 ± 0.28 mg /100g) and T2 (0.34 ± 0.03 mg/kg and 8.6 ± 0.29 mg/100g). Aflatoxin residues were detected in 10% and 30% of muscle and liver samples in the control group with limits 2.22 ± 0.09 and 5.13 ± 0.12 µg/kg, respectively. Meanwhile, it was detected in 15% and 10% of the examined liver of T1 and T2, respectively. T1 and T2 supplementation improves growth rate, haematological and meat quality parameters while decreasing aflatoxin residues in the liver and muscle.

**Key words:** broiler meat; aflatoxin residues; growth parameters; meat quality; thiobarbituric acid; total volatile basic nitrogen

### Introduction

The consumption of chicken meat products increased worldwide due to the high content of essential amino acids, and their competitive price. Chicken meat is considered a significant source of protein in Egypt due to the lack of red meat production (1). Many crops utilized as primary chicken feed ingredients, such as maize, peanut

meal, cottonseed meal, and sorghum, are prone to mycotoxin contamination, posing a higher risk of mycotoxin introduction into poultry rations (2). Mycotoxins, particularly aflatoxins, are frequently found in chicken feed, posing a health and productivity risk. Mycotoxin residues in chicken products may also pose a risk to humans due to their carcinogenic, mutagenic, teratogenic, immunosuppressive, and other negative consequences (3).

Furthermore, mycotoxins contaminate many of the crops, as well as about 25% of the world's food supply, on an annual basis (4).

*Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, are the incriminated molds in aflatoxin production under certain conditions. Aflatoxin B1 (AFB1), the most toxic and well-known carcinogen (5), is listed in Group 1 of carcinogenic agents by the International Agency for Research on Cancer (IARC) (6). Previous investigations have extensively reported the presence of probable aflatoxin producing strains and in commercial chicken ration and chicken product (7; 8). Producers, researchers, and governments, on the other hand, are working to create effective preventative management and decontamination methods to reduce the hazardous consequences of aflatoxin in animal husbandry. Using a mycotoxin binder, a method has been created to decrease and eradicate the impact of mycotoxin. Mycotoxin binder is a feed additive that has no nutritional value but can bind mycotoxin in the digestive tract, allowing it to be expelled in the faeces. Studies on adsorbent-based techniques to remove mycotoxins from contaminated feed and reduce their impact on poultry health have been conducted since the early 1990s (9). Different synthetic zeolites and a natural sodium bentonite (Na-B) were tested for their capacity to bind aflatoxin in earlier research. The protective efficacy of these adsorbents for aflatoxicosis prevention in chicken was proven in both in vitro and in vivo studies (10;11). Sepiolite is a form of mycotoxin binder that has been found to mitigate the detrimental effects of aflatoxin in laying hens by increasing egg production and lowering the feed conversion ratio (12). Enzymes or microorganisms are used in biological techniques to bio transform mycotoxins into less hazardous metabolites. De-epoxidases are enzymes produced by the *Coriobacteriaceae* family that open the hazardous epoxide ring of trichothecenes, such as DON and T-2 toxin, and thereby detoxify them (13). In accordance with Regulation (EC) No 1831/20031, which specifies the rules governing the community authorization of additives for use in animal nutrition (14), EU regulations have been developed addressing enzyme fumonisin esterase as a feed supplement for pigs and poultry. The study was conducted to evaluate the effect of

two commercial antimycotoxins on the growth rate, blood parameters, broiler meat quality, and aflatoxin residues on challenged broilers with natural aflatoxin in rations.

## Materials and methods

### *Case history and experimental design*

Mycotoxicosis has been reported in broiler flocks at a station in Sharika province on several occasions. As a result, the birds suffered from body weight loss, vaccination failure, liver and kidney enlargement, and liver subcapsular haemorrhage. Standard broiler ration with  $\geq 36 \pm 0.21$   $\mu\text{g}/\text{kg}$  total aflatoxin. Ninety birds of Avian48 were submitted to experimental procedures in a trial to eliminate the aflatoxicosis problem in the bird station. They were housed in one deep litter pen that was divided into 3 groups (30 birds each), bedded with wood shavings. The first as control group fed ad libitum (15) on standard broilers ration naturally contained  $36 \pm 0.21$   $\mu\text{g}/\text{kg}$  total aflatoxin, the second group fed on the aflatoxin contaminated ration with the addition of (Bentonite 55.6% - Sepiolite 44.4% obtained from KEMIN, Belgium) by inclusion rate (1kg /ton) treatment one (T1). The third group fed on aflatoxin contaminated ration with the addition of (Bentonite 65%-*Coriobacteriaceae*  $0.85 \times 10^{11}$  CFU- fumonisin esterase 7500 U obtained from MIAVIT, Germany) by inclusion rate (500 gm/ton) treatment two (T2). Both T1 and T2 introduced to ration according to EFSA (10). The lighting program was 24 h light with temperature maintained between 25 and 32 °C.

### *Growth parameters*

The body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), Cumulative feed intake (CFI) and feed conversion rate (FCR) was calculated according to Mukan dungutse et al. (16) on the 21, 28 and 35 days.

### *Hematological parameters*

A vein puncture was used to collect two blood samples. The first blood sample (0.5mL) was drawn on EDTA disodium salt and used for hematological analysis. The serum biochemical assays were determined using the second blood sample (3ml), which was collected in a centrifuge tube to separate serum.

**Table 1:** Ingredients of the basal starter, grower, and finisher rations fed to broilers

Ingredients (%)	Starter	Grower	Finisher
Yellow corn, ground	54.45	60.18	65.71
Soybean meal, 46% CP	37.22	28.75	21.82
Corn gluten meal, 60% CP	2.23	4.62	5.74
Soybean oil	2.02	2.33	2.80
Limestone	0.93	1.03	0.82
Dicalcium phosphate	1.88	1.81	1.74
Salt (NaCl)	0.26	0.27	0.27
DL- Methionine	0.17	0.14	0.12
L- Lysine HCl	0.24	0.28	0.38
Threonine	0.96	0.87	0.79
Vitamins and minerals mixture	3.00	3.00	3.00

The Neubauer hemocytometer was used to perform erythrocytic and total leukocytic counts, with Natt and Herrick solutions as special diluents (17). The microhematocrit centrifuge was used to estimate the packed cell volume (PCV) value. The cyanmethemoglobin colorimetric method was used to estimate haemoglobin (Hb) concentration (18). The researchers calculated the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). The differential and absolute counts of leukocytic cells were performed. (17).

#### *Meat quality parameters*

The broilers were slaughtered in a small-scale poultry processing plant at 35 days of age in competence with the regulation of Egyptian Ministry of Agriculture. Meat quality analysis was carried out on the 3<sup>rd</sup> day of storage at chilling temperature  $3\pm 1^{\circ}\text{C}$ . Water holding capacity of 1 g chicken meat sample was measured by centrifuging at 1500 g for 4 min and drying at  $70^{\circ}\text{C}$  (19). A CP-411 pH-meter (Elmetron, Zabrze, Poland) was used to measure the pH of meat samples (1). Two buffers of 4 and 7pH were used to calibrate the electrode. Five grammes of muscle were pulverized twice in a meat grinder and carefully mixed to ensure sample homogeneity, and then the pH was determined. TVB-N was established (20). In a blender, ten grammes of chopped broiler fillet sample were homogenized with 100 mL distilled water for two minutes. After washing the sample with 200 mL of water, 2 g of magnesium oxide, and 2 drops of antifoaming agent were added to the distillation flask. In a 500 mL flask, the mixture was allowed to boil for 10 minutes before being distilled for around 25

minutes at the same rate into 25 mL of 2% boric acid solution with a few drops of screened methyl red indicator. The condenser was washed down with distilled water once the heating was turned off. The contents of the flask and the blank solution (25 mL of 2% boric acid) were titrated with 0.1 N H<sub>2</sub>SO<sub>4</sub> (titer), and TVB-N (mg N/100 g flesh) was calculated using the formula TVB-N = 14 (titer–blank). Ten grammes of broiler fillet were mixed with 25 mL of 20% trichloroacetic acid (w/v) and homogenized in a blender for 30 seconds to calculate TBARS (21). Following filtration, 2 mL of the resulting solution was added to 2 mL of 0.02 mL aqueous TBA and incubated for 20 hours at room temperature in the dark. A UV–vis spectrophotometer (model UV-1200, Shimadzu, Japan) was used to detect the absorbance at 532 nm. TBARS levels were measured in milligrams of malondialdehyde (MDA) per kilogram of chicken meat.

#### *Aflatoxin residues*

Total aflatoxins (B1+B2+G1+G2) estimated by fluorometer (VICAM. Series 4, USA) twenty-five grams of ground samples with five grams of NaCl were extracted in hundred mL methanol: water (80:20) three times. The extracts were diluted four times with bi-distilled water and filtered using glass microfibre filter, only four mL of filtrated extract at a rate of 1-2 drops/second passed through (AflaTest®-P affinity column, VICAM, USA). One mL of HPLC grade methanol (Merck, Germany) was used for elution of affinity column at a rate of one to two drops/second and finally collected in a glass cuvette. The AflaTest developer added 0.1 mL and mixed it

well with the content of the cuvette then put the mixture in calibrated fluorometer followed by a reading of aflatoxin level after 1 min. The limit of detection ranged from 0.1 ppb to 300 ppb according to Abd-Elghany and Sallam (22).

#### *Statistical analysis*

SPSS for Windows was used to examine the data. The data is presented as a mean with standard error (SE). For multiple comparisons, the significance of differences was assessed using one-way analysis of variance (ANOVA) and the Duncan procedure. Any  $P < 0.05$  value was considered significant. Means in the same column with different letters differed considerably and highest value was represented with letter a (23)

## **Results**

### *Growth parameters*

Aflatoxin significantly reduced ( $p < 0.05$ ) growth parameters such as body weight gain and average daily gain in the positive control group compared to the treated groups T1 and T2 at 21-, 28-, and 35-days of age. In the meantime, had no effect on average daily feed intake or cumulative feed intake. On 21, 28, and 35 days, the mean value of feed conversion rate in the control group was  $1.63 \pm 0.08$ ,  $1.74 \pm 0.15$  and  $1.88 \pm 0.13$ , which was significantly higher ( $p < 0.05$ ) than (T1)  $1.36 \pm 0.05$ ,  $1.53 \pm 0.10$ ,  $1.52 \pm 0.09$  and (T2)  $1.29 \pm 0.04$ ,  $1.54 \pm 0.04$ ,  $1.56 \pm 0.08$  on 21-, 28- and 35-days age, respectively (Table 2).

### *Haematological parameters*

The results in (Table, 2) showed that haematological parameters such as RBCs count, Hb concentration and PCV value significantly de-

creased ( $p < 0.05$ ) in control group compared with T1 or T2 in T2 which significantly higher ( $p < 0.05$ ) than control and T1. In contrast, the MCV value in the control was significantly higher ( $p < 0.05$ ) than both T1 and T2. On comparing MCH - and MCHC in control and treated groups, no revealed significant difference. The total leucocytic count in descending manner  $23.3 \pm 0.44 \times 10^3$ ,  $20.73 \pm 0.2 \times 10^3$  and  $18.3 \pm 0.65 \times 10^3/\mu\text{l}$  in T1, T2 and control group, respectively. The control group significantly lower ( $p < 0.05$ ) in total leukocytic count, heterophils, lymphocytes and monocytes than T1 and T2. Meanwhile, no significant difference in eosinophils or basophils count in all groups as shown in Table (3).

### *Meat quality parameters*

The water holding capacity after 3 days chilling at  $3 \pm 1^\circ\text{C}$  was greatly affected in the control group. It was  $56.64 \pm 1.23$  versus, T1 and T2 were,  $61.23 \pm 1.34$  and  $62.12 \pm 1.33\%$ , respectively. Both of T1 and T2 were significantly higher in (WHC) ( $p < 0.05$ ) than in the control group Figure (1. A). The pH mean values were  $6.22 \pm 0.14$ ,  $6.14 \pm 0.12$  and  $6.11 \pm 0.13$  in the control, T1 and T2, respectively. There were no significant differences in pH between examined groups Figure (1. B). The TBA mean values in the control group was  $0.45 \pm 0.05$  mg/kg while, in T1 and T2 mean values were  $0.37 \pm 0.04$  and  $0.34 \pm 0.03$  mg/kg, respectively. The control group was significantly higher ( $p < 0.05$ ) than both T1 and T2 (Figure 1. C). Total volatile nitrogen after 3 days chilling at  $3 \pm 1^\circ\text{C}$  was significantly higher ( $p < 0.05$ ) in the control group ( $11.2 \pm 0.35$  mg/100g) on comparison with T1 ( $9.6 \pm 0.28$  mg/100g) and T2 ( $8.6 \pm 0.29$  mg/100g) Table (1. D).

**Table 2:** Effect of different treatments on body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), cumulative feed intake (CFI) and feed conversion rate (FCR) (Mean  $\pm$  SE) (n=20 of each group)

Age	Groups	BWG	ADG	ADFI	CFI	FCR
21 days	Control	590 $\pm$ 19.69 <sup>b</sup>	56.04 $\pm$ 0.76 <sup>b</sup>	91.6 $\pm$ 4.39 <sup>a</sup>	964.6 $\pm$ 30.34 <sup>a</sup>	1.63 $\pm$ 0.08 <sup>a</sup>
	T1	717.6 $\pm$ 17.92 <sup>a</sup>	64.5 $\pm$ 1.54 <sup>a</sup>	88.2 $\pm$ 3.7 <sup>a</sup>	980 $\pm$ 44.72 <sup>a</sup>	1.36 $\pm$ 0.05 <sup>b</sup>
	T2	712 $\pm$ 20.19 <sup>a</sup>	65.5 $\pm$ 0.71 <sup>a</sup>	85 $\pm$ 4.06 <sup>a</sup>	922 $\pm$ 9.60 <sup>a</sup>	1.29 $\pm$ 0.04 <sup>b</sup>
28 days	Control	956 $\pm$ 78.21 <sup>b</sup>	34.26 $\pm$ 1.63 <sup>b</sup>	121.2 $\pm$ 2.86 <sup>a</sup>	1666 $\pm$ 55.05 <sup>a</sup>	1.74 $\pm$ 0.15 <sup>a</sup>
	T1	1124 $\pm$ 83.85 <sup>a</sup>	39.88 $\pm$ 3.12 <sup>a</sup>	122.4 $\pm$ 3.58 <sup>a</sup>	1724 $\pm$ 24.08 <sup>a</sup>	1.53 $\pm$ 0.10 <sup>b</sup>
	T2	1071 $\pm$ 26.08 <sup>a</sup>	37.48 $\pm$ 0.12 <sup>a</sup>	117 $\pm$ 3.87 <sup>a</sup>	1660 $\pm$ 34.09 <sup>a</sup>	1.54 $\pm$ 0.04 <sup>b</sup>
35 days	Control	1403 $\pm$ 57.4 <sup>b</sup>	39.87 $\pm$ 2.24 <sup>b</sup>	148.6 $\pm$ 7.64 <sup>b</sup>	2648 $\pm$ 120.08 <sup>a</sup>	1.88 $\pm$ 0.13 <sup>a</sup>
	T1	1748 $\pm$ 72.94 <sup>a</sup>	46.52 $\pm$ 1.78 <sup>a</sup>	162.6 $\pm$ 7.5 <sup>a</sup>	2669 $\pm$ 80.34 <sup>a</sup>	1.52 $\pm$ 0.09 <sup>b</sup>
	T2	1681 $\pm$ 86.05 <sup>a</sup>	43.26 $\pm$ 2.71 <sup>a</sup>	163.6 $\pm$ 9.91 <sup>a</sup>	2624 $\pm$ 34.07 <sup>a</sup>	1.56 $\pm$ 0.08 <sup>b</sup>

Means in the same column with same examination day different superscript letters (a, b and c) are significantly different ( $P < 0.05$ )

**Table 3:** Effect of different treatments on haematological parameters (Mean  $\pm$  SE) on 35 days old broiler (n=20 of each group)

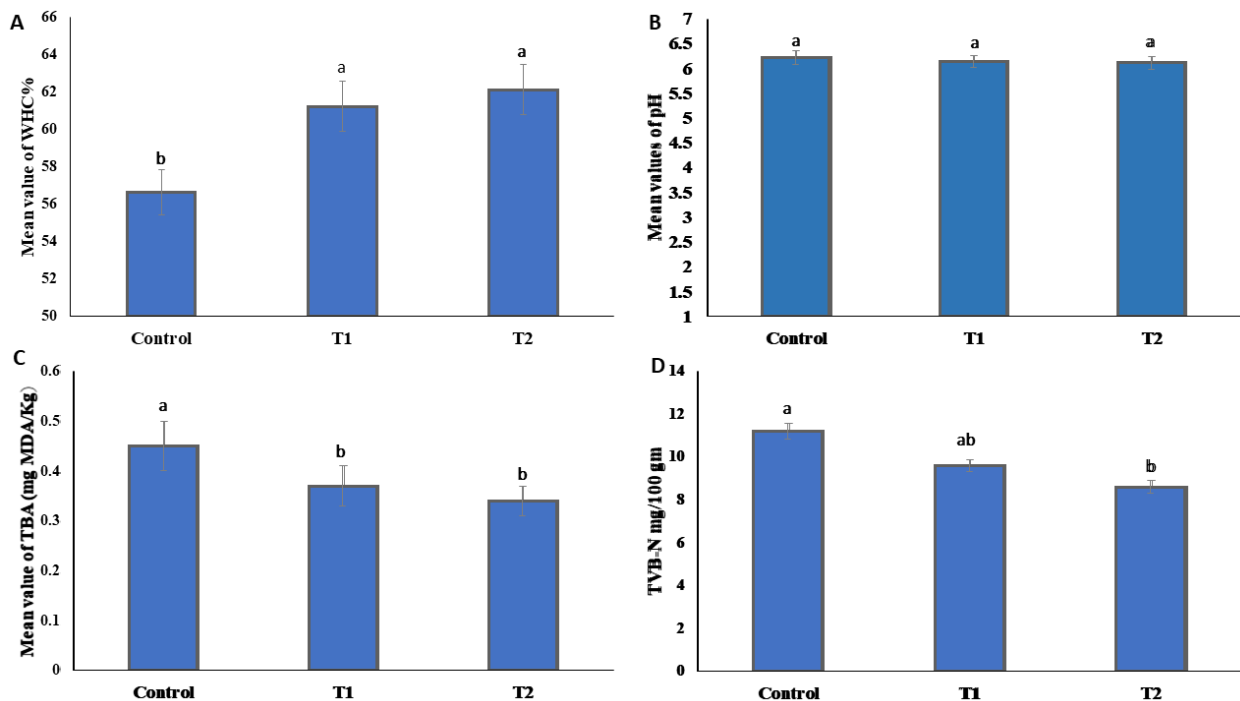
Groups	RBCs $\times 10^6/\mu\text{l}$	Hb Gm%	PCV %	MCV Fl	MCH Pg	MCHC%
Control	2.22 $\pm$ 0.08 <sup>b</sup>	8.08 $\pm$ 0.11 <sup>b</sup>	23.7 $\pm$ 0.63 <sup>c</sup>	105.9 $\pm$ 3.4 <sup>a</sup>	35.1 $\pm$ 1.1 <sup>a</sup>	33.9 $\pm$ 0.66 <sup>a</sup>
T1	2.82 $\pm$ 0.11 <sup>b</sup>	9.33 $\pm$ 0.30 <sup>ab</sup>	27.8 $\pm$ 0.54 <sup>b</sup>	98.7 $\pm$ 2.14 <sup>b</sup>	32.9 $\pm$ 0.41 <sup>a</sup>	33.6 $\pm$ 0.51 <sup>a</sup>
T2	3.29 $\pm$ 0.07 <sup>a</sup>	10.48 $\pm$ 0.25 <sup>a</sup>	30.74 $\pm$ 0.79 <sup>a</sup>	93.3 $\pm$ 2.75 <sup>b</sup>	31.8 $\pm$ 0.42 <sup>a</sup>	34.2 $\pm$ 0.75 <sup>a</sup>

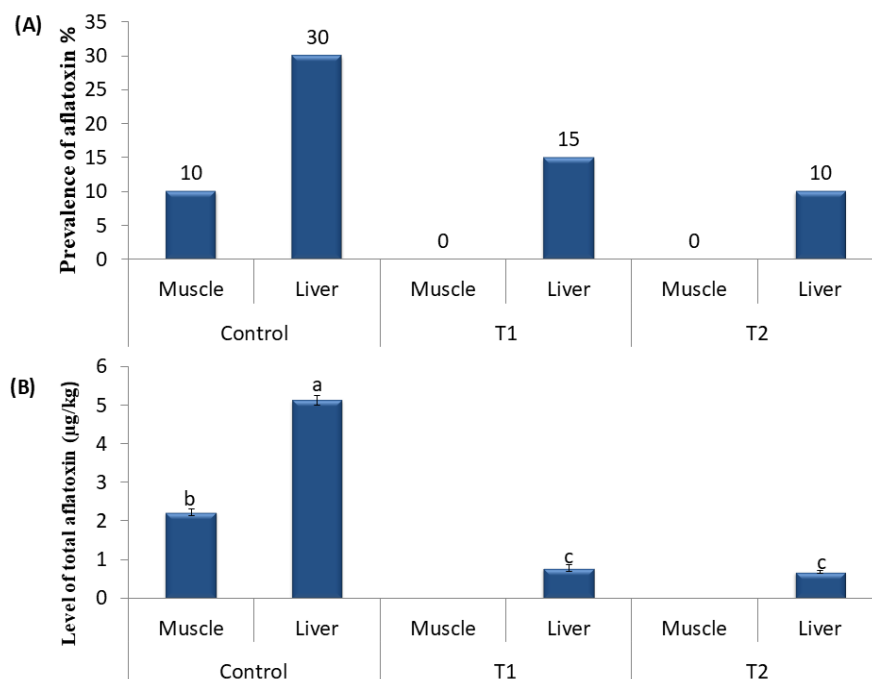
Means in the same column with different superscript letters (a,b and c) are significantly different (P<0.05)

**Table 4:** Effect of different treatments on differential leukocytic count (Mean  $\pm$  SE) on 35 days old broiler (n=20 of each group)

Group	WBCs ( $\times 10^3/\mu\text{l}$ )	Differential leukocyte count ( $\times 10^3/\mu\text{l}$ )				
		Heterophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Control	18.3 $\pm$ 0.65 <sup>c</sup>	6.26 $\pm$ 0.05 <sup>c</sup>	8.90 $\pm$ 0.40 <sup>c</sup>	1.39 $\pm$ 0.04 <sup>a</sup>	1.57 $\pm$ 0.05 <sup>b</sup>	0.18 $\pm$ 0.07 <sup>a</sup>
T1	20.73 $\pm$ 0.2 <sup>b</sup>	6.88 $\pm$ 0.12 <sup>b</sup>	10.48 $\pm$ 0.18 <sup>b</sup>	1.46 $\pm$ 0.11 <sup>a</sup>	1.75 $\pm$ 0.04 <sup>ab</sup>	0.17 $\pm$ 0.08 <sup>a</sup>
T2	23.3 $\pm$ 0.44 <sup>a</sup>	7.58 $\pm$ 0.38 <sup>a</sup>	12.11 $\pm$ 0.14 <sup>a</sup>	1.49 $\pm$ 0.05 <sup>a</sup>	1.9 $\pm$ 0.08 <sup>a</sup>	0.22 $\pm$ 0.8 <sup>a</sup>

Means in the same column with different superscript letters (a,b and c) are significantly different (P<0.05)

**Figure 1:** Effect of different treatment on meat quality parameters (A) Mean value of water holding capacity (WHC). (B) Mean value of pH (C) Mean values of thiobarbituric acid (mg malonaldehyde/ kg). (D) Mean values of total volatile basic nitrogen (TVB-N mg/100 g)



**Figure 2:** Effect of different treatments on (A) prevalence of total aflatoxin residues muscle and liver. (B) level of aflatoxin residues ( $\mu\text{g}/\text{kg}$ ) muscle and liver (Mean  $\pm$  SE) ( $n=20$ )

#### *Aflatoxin residues*

Aflatoxin residues were detected only in 2/20 (10%) of muscle samples, whereas they were detected in 6/20 (30%) of liver samples after 35 days of feeding on contaminated ration in the control group. The aflatoxin residue was below the detection limit in muscle samples and only detected in 3/20 (15%) and 2/20 (10%) of the examined liver of T1 and T2, respectively Figure (2.A). The level of aflatoxin residues was  $2.22 \pm 0.09$  and  $5.13 \pm 0.12$   $\mu\text{g}/\text{kg}$  of examined muscle and liver of the control group. The level was significantly reduced ( $P < 0.05$ ) in livers of T1 ( $0.78 \pm 0.08$   $\mu\text{g}/\text{kg}$ ) and T2 ( $0.68 \pm 0.08$   $\mu\text{g}/\text{kg}$ ) Figure (2. B).

#### **Discussion**

Aflatoxin has the greatest negative impact on bird production in terms of weight gain, feed consumption, feed conversion ratio (FCR), and harvest (24). Feeding on aflatoxin contaminated ration significantly reduced ( $p < 0.05$ ) BWG and ADG in the control group than T1 and T2 on 21-, 28- and 35-days age Table (1). The finding may be attributed to the interference of aflatoxin with carbohydrate, fat, and nucleic acid metabolism in livestock (25). Regarding to the effect of bentonite in T1 and T2 comparable results obtained by

Magnoli et al (26) who found that aflatoxin contaminated ration decreased growth performance of chicken and this effect was ameliorated by the addition of bentonite to ration. Also, Sepiolite significantly increased organic matter digestibility, which corresponded to retention time in the gut of poultry, thus slowing the rate of digesta passage would allow endogenous enzymes to be more effective in the digestion of carbohydrates, fat, and protein (27). The *Coriobacteriaceae* in T2 has the capacity to reduce mycotoxin from contaminated feed and proved as a feed additive for all poultry species and amending Implementing Regulation (28). The effect of fumonisin esterase in T2 explained previously in five short-term feeding studies were conducted in which poultry were fed fumonisin-contaminated feed with or without the additive. The enzyme was able to significantly reduce the concentration of fumonisin in faeces and various points in the digestive tract while increasing the concentration of the degradation products (29).

Aflatoxins cause economic losses to broiler besides affecting the healthy state of birds and consumer. The haematological parameters such as RBCs count, Hb concentration and PCV value significantly decreased ( $p < 0.05$ ) in the control group. This decrease in the hematological parameters may

be due to many factors such as inhibition of protein synthesis as evidenced by lower serum albumin, decrease of the total iron binding capacity, and the hemopoietic cellular defects of aflatoxin (30). Keçeci et al. (31) have reported that the hematocrit and hemoglobin levels, MCH, and thrombocyte counts were depressed in broilers feed on aflatoxin contaminated ration, and Oğuz et al. (32) reported that hematocrit, hemoglobin levels, MCV, erythrocyte, thrombocyte, and lymphocyte counts decreased due to exposure to aflatoxin in ration. The immunosuppressive impact of aflatoxin has been linked to the fact that it inhibits protein synthesis directly (30). The control group significantly lower ( $p < 0.05$ ) in total leukocytic count, heterophil, lymphocyte, and monocyte than T1 and T2. Previous reports of lymphocytopenia and monocytopenia in chicken fed on aflatoxin contaminated ration (30;32). The inclusion of bentonite was found to be effective in reducing the unfavorable effect of aflatoxin on leukocyte and lymphocyte production in the current investigation. The activity of Na-bentonite as a sequestering agent against aflatoxin in the diet by lowering its bioavailability in the gastrointestinal tract could explain this effect (33).

The nutritional value of the meals we eat is becoming increasingly important to consumers. Along with nutritional composition, pH and water holding capacity are crucial meat quality evaluation characteristics that can help ensure that the end product is of exceptional quality and profitability. (34). WHC is a significant meat quality characteristic. In the muscle, about 88 to 95 percent of the water is retained intracellularly in the gap between actin and myosin filaments (35). The WHC in our study was in the same line with Shabani et al, (36) who found chicken fed on aflatoxin-contaminated ration without addition of nanozeolite had the lowest WHC. The postmortem conversion of muscular glycogen stores to lactic acid, which accumulates in muscles, affects the pH of the meat (37). In the current study, pH value revealed no significant differences between control and treated groups such finding noticed previously (36).

Lipid oxidation has been linked to poor look, flavour, and texture in poultry meat. Endogenous prooxidants in muscle, such as myoglobin and ionic

irons, are tightly regulated by a variety of endogenous antioxidant agents, including reducing chemicals (38). Results of the current study show the increase of TBA levels, which has been previously obtained by Shabani et al (36). They found that chicken fed with aflatoxin contaminated ration had significantly more TBA levels.

The obtained results revealed control group had significant higher ( $p < 0.05$ ) TBA than both treated groups which may be due to presence of aflatoxin that act as a free radical creator in control group. As previously stated, aflatoxin inhibits protein synthesis, resulting in a decrease in the concentration of produced ceruloplasmin and transferrin in the liver. Increased free copper and iron ions in organs result in changes in the immune system's response to lipid peroxidation because of this reduction. In reaction to fentons, a lipid preoxidation phase, iron plays a key role (39). The lowering of free iron levels caused by banding iron from outside and inside absorption functions as a mechanism to avoid lipid peroxidation (40). According to Eraslan et al. (41), aflatoxin may cause interference in compounding iron and internal absorbers. As a result of liver damage and protein synthesis failures, free iron ions rise. Total volatile basic nitrogen (TVB-N), an important index that represents the concentration of ammonia, trimethylamine, and dimethylamine in measured samples, has long been used to determine the quality of meat and meat products (42). Significantly, higher level of TVB-N in control group ( $P < 0.05$ ) may be due to compromised immunity, which enhances migration of spoilage bacteria from gastrointestinal tract to muscle to degrade protein. Aflatoxin identified as a main immunosuppressive agent (43).

The major problem caused by aflatoxin contamination in broiler production is not the economic loss due to poor growth rate, but aflatoxin, which is readily transferred to human diet. Clearly, this toxin cannot be seen with the naked eye, necessitating the use of specialized testing to determine the potential damage to human health (44). In this study, aflatoxin detected with high rate in livers 30% and limit  $5.13 \pm 0.12 \mu\text{g}/\text{kg}$  in control group. This explained where, liver is the primary organ responsible for processing mycotoxins, detoxifying them, and protecting the body from their toxic effects. The liver is a vital organ for the metabolism and utilization of lipids, proteins, and amino acids (44). Comparable results obtained in

China broilers fed on ration containing 40 µg/kg aflatoxin for 21 days (45) and in Thailand, broilers fed on ration containing 40 µg/kg aflatoxin 50 µg/kg (46) aflatoxin for 28 days aflatoxin residues were 11.48 and 0.40 µg/kg liver, respectively.

In experimental report Aflatoxin residues were significantly reduced in the bird liver and kidney tissues in the instance of the two antimycotoxin binders (47). The concentration of aflatoxin is not the only major factor that influences the effect of aflatoxin and its accumulation in edible poultry parts; the fungal strain that produces the toxin may be the most important factor. The effect of T1 and T2 on reducing the level of aflatoxin residues explained where electron donor acceptor mechanism is the dominant in dry conditions, while the formation hydrogen bond between active sites on the aflatoxin molecule and bentonite is the most important model during wet conditions in bird intestine. Bonding between carbonyl groups in aflatoxin and active sites on bentonite (exchangeable cations in inter-layer space) to absorb toxin on bentonite (48). In addition, sepiolite is a phyllosilicate mineral that has a sheet-like structure. Because sepiolite has such a huge surface area, aflatoxin binding capacity may be considerably higher. In aflatoxin molecules, sepiolite functions as a carbonyl group binder (49). Aflatoxin binding prevents absorption as well as accumulation in broiler liver and muscle.

## Conclusion

Broiler feed contaminated with aflatoxin has a bad effect on growth parameters while having a negative effect on haematological parameters. The meat quality parameters decreased after 3 days of chilling, predicting rapid spoilage of broiler meat and an increase in the possibility of aflatoxin residues in muscle and liver. The use of T1 or T2 altered the effect of aflatoxin in the ration and enhances the protection of human health against aflatoxin residues.

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