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TOXICOLOGICAL EFFECTS OF AFLATOXIN B1 ON GROWTH PERFORMANCE, HUMORAL IMMUNE RESPONSE AND BLOOD PROFILE OF JAPANESE QUAIL

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ABSTRACT

Toxic effects of Aflatoxin B1 on growth performance and humoral immune response against Newcastle disease Virus (NDV) vaccine were determined in Japanese quails. Quail chicks (n=300) were divided into five groups and each group was further sub-divided into two subgroups. One subgroup of each group was vaccinated with NDV vaccine to study the antibody response. Experimental diets were prepared by adding different levels of AFB1 i.e. 0.25mg/kg feed, 0.5mg/kg feed, 1 mg/kg feed and 2 mg/kg feed, and were offered to groups B, C, D and E respectively for 42 days, while group A was kept as control. Feed intake, body weight gain and feed conversion ratio were calculated on weekly basis. The Antibody titers of vaccinated quails were measured by haemagglutination inhibition test. Hematological parameters Erythrocyte sedimentation rate, Hb content, hematocrit and total leukocyte count were measured in AFB1 fed birds and were compared with the quails given basal diet. Results showed significant reduction ($P \leq 0.05$) in feed intake, weight gain and feed conversion ratio, when compared to control group. Moreover, anti-NDV antibody titre was significantly reduced *($P \leq 0.05$)* in AFB1 fed groups. Hb content, hematocrit, and TLC had time and dose dependent decrease in AFB1 fed quails while ESR was increased significantly in AFB1 fed birds. This highlighted adverse effects of AFB1 on growth performance, humoral immune response and blood profile of quails.

Keywords: Aflatoxin B1, Japanese quails, growth performance, humoral immune response, blood profile.

INTRODUCTION

Aflatoxin contamination in agricultural commodities is now well recognized as a public health hazard (Reddy *et al*. 2010). The main factors responsible for the mycotoxins production are improper control of moisture content and temperature (Singh and Mandal, 2014). Aspergillus species, common toxigenic moulds, are widely distributed in air, soil, organic materials and plant parts all over the world. Among these, three species *Aspergillusflavus, A. parasiticus* and *A. nomius* have received major consideration due to their ability of producing potent toxins (Jia *et al.* 2016). Mycotoxins are found in feed as secondary metabolites produced by mold during their life span and are serious worldwide problem with high economic impact (Pappas *et al*. 2016).Crops contaminated with Aflatoxins are a worldwide problem and approximately 25% of world's food supply is contaminated with Mycotoxins annually (CAST, 1989). Aflatoxins decrease resistance to diseases and suppress vaccine-induced immunity in birds (Diekman and Green, 1992). Oguz *et al.,(*2003) reported that Aflatoxins are hepatotoxic, hepatocarcinogenic, teratogenic, mutagenic and immunosuppressive.

The most potent toxin is aflatoxin B1 produced by Aspergillus flavus (Abousadi *et al*. 2007). The effects of Mycotoxin on higher animals include hepatotoxicity, nephrotoxicity, immune toxicity, oncogenesis and genotoxicity (Shareef, 2010). Aflatoxins at levels of parts per billion (ppb) is thought to impart substantial losses to livestock industry (Bhat and Vasanthi, 2003).

Aflatoxin contamination in poultry feed affects animal health, production, biochemical and hematological parameters, changes in gene expression of liver enzymes, liver damage, kidney abnormalities, mortality and immunosuppression, which may enhance susceptibility to infectious diseases (He *et al*, 2013). Susceptibility to aflatoxins can vary considerably between different poultry species (Arafa *et al*. 1981).

The adverse effects of aflatoxins vary according to the dose, natural or pure aflatoxins, the duration of exposure, and animal factors such as age, sex, and level of stress (Whitlow and Hagler, 2005).

The available data on aflatoxin toxicity in quail, however, are mainly derived from short-term studies using high levels of toxins in feed, without specifying any of four naturally occurring aflatoxins. In Pakistan, most of the research work has been done on toxicogenic fungi in feed and feed ingredients and their aflatoxin level (Afzal *et al*., 1979). No work has been done on experimental aflatoxicosis in quail using different toxin levels. Hence, present study was designed to investigate the toxicological effects of graded doses of aflatoxin B1 on growth performance, humoral immunity and blood profile of quails under experimental conditions.

MATERIALS AND METHODS

The research model was based on a complete randomized design. Japanese quail chicks (n=300) were procured from Avian Research and Training Centre, University of Veterinary and Animal Sciences, Lahore. Quail chicks were randomly divided into five groups, each containing sixty chicks. Each group was further subdivided into two sub-groups.

Aflatoxin B1 Production: *Aspergillus flavus* procured from the department of Microbiology UVAS, Lahore and confirmed on the basis of macroscopic and microscopic culture characteristics as described by Rodrigues *et al.* (2009). Aflatoxin was produced by the culturing of *Aspergillusflavus* on rice, wheat and maize grains with the slight modification in a protocol described by Shotwell *et al*., 1966. Presence of Aflatoxin B1 in powder samples (n=3) was checked by Thin Layer Chromatography (TLC) following the protocols described by Trucksess *et al*., 1994.

Aflatoxin B1 Feeding: Basal diet without aflatoxin B1 was fed to control quails (group A). Aflatoxin B1 added in the diet of experimental groups for period of 6 weeks at dose rate of 0.25 mg/kg feed (group B), 0.50mg/kg feed (group C), 1.0mg/kg feed (group D) and 2.0mg/kg feed (group E) as described by Simsek *et al*., 2007. One sub-group of each group was vaccinated with Newcastle Disease Virus (NDV) vaccine for the evaluation of humoral immune response. Weekly average body weight and weekly weight gain of birds were recorded throughout the study period including the day-old chick weight. Quails were observed for 42 days to check the effects of aflatoxin B1 on growth performance parameters including body weight gain, feed intake and feed conversion ratio. Weekly weight gain was calculated at the end of each week by using the formula (weight gain = final weight – initial weight). A weekly record of feed intake was kept for each replicate during the experiment by using the formula (Feed intake = feed offered – feed refused). Feed conversion ratio was calculated by using the formula (FCR = feed intake in grams / body weight gain in grams).

Hematology: At the end of each week, five (05) quails from each treatment were randomly selected, weighed and killed by cervical dislocation. Blood samples were taken by cardiac puncture in anticoagulant coated tubes for hematological parameters and in serum storage tubes for immunological studies as described by Mangoli *et al*., 2011. Hemoglobin, packed cell volume and white blood cell count were estimated by method as described by Oguz, *et al*., 2000. Erythrocyte Sedimentation Rate (ESR) was determined by Westergren Method (Westergren, 1957). For measuring humoral immune response, serum samples were tested for anti-NDV antibodies using haemagglutination inhibition test (Maxwell *et al*., 2005).

Statistical Analysis: The results were recorded and statistically compared by using SPSS version 16.0 applying two-way ANOVA followed by Duncan's Multiple Range Test at 0.05 level of significance. (SPSS, 1988).

RESULTS AND DISCUSSION

Results of this debut study on toxicological effect of AFB1 on Japanese Quails kept under experimental conditions revealed that the feed intake (grams) was statistically lower (P<0.05) in aflatoxin treated groups. The feed intake was significantly reduced (P<0.05) in aflatoxin fed groups. Comparison within groups showed significant reduction in feed intake in all the toxin ingesting birds. Similar patterns were observed in group B and C. Group D and E showed highest reduction in feed intake which is attributed to the higher levels of toxin intake (Table - 1). Similarly, the decreased in body weight gain (grams) was observed. It was dose and time dependent. As the dose of Aflatoxin B1 was increased, the body weight gain within the groups were decreased significantly (P<0.05). When compared within groups, weekly weight gain in group B decreased significantly (P<0.05) in second week throughout the end of study period. Similar pattern was observed in rest of the experimental groups when compared within groups. Group fed with 2mg/kg of AFB1 showed maximum drop in weight gain which is due to high dose of toxin ingested. (Table – 2). No mortality was recorded in any of treated groups. The feed conversion ratio was statistically altered by the ingestion of AFB1. The poor FCR was due to the decreased feed intake. It was observed that growth performance parameters were dose and duration dependent. As the dose of Aflatoxin B1 was increased, the quails with in the groups showed significant reduction in feed conversion ratios. The group D (1mg AFB1/kg feed) and E (2mg AFB1/kg feed) showed poor feed conversion ratio as compared to the other treated groups When compared within groups FCR was significantly reduced (P<0.05) after a week of toxin intake in all the intoxicated groups. FCR was lowest in group E and F throughout the study period and was lowest in fifth and sixth week (P<0.05). (Table - 3). At higher level of toxin in feed adverse change in the body weight gain was observed. Heba and Hesham (2004) reported that aflatoxin significantly decreased the feed intake and body weight gain and had negative effects on feed conversion ratio in broiler chicken which is in agreement with the present study. Doerr *et al.* (1983) reported that minimum level of aflatoxins needed to cause the deleterious effects on the performance of the broiler chicken is 2.7 mg /kg feed. In the present study group E was given 2mg AFB1 /kg feed showed statistically significant reduction on growth performance of the quail chicks. The most prevalent effect of aflatoxin toxicity is reduced body weight gain which greatly affects the poultry industry. In the present study the feeding 2mg AFB1 / kg feed significantly decreased the body weight gain and feed intake in quails from 14 to 42 day of age as compared with the control group. Aflatoxin B1 also had adverse effects on the feed conversion ratio, these finding are in accordance with Kubena *et al*. (1998). Oguz and Kurtoglu (2000) reported the similar results in broiler chicks when given 2.5 mg AF/kg diet for 3 week. The findings of the present studies are also agreement with the report of experimental aflatoxicosis in quails by Parlat *et al.* (1999) and Sadana *et al*. (1992). The deleterious effects of aflatoxin B1 on the growth performance may be due to anorexia, listlessness, impaired general health and the inhibitory effects of aflatoxin B1 on protein synthesis and lipogenesis (Kiran *et al*., 1998). The result of the present study are agreement with the finding of Xin-Yan *et al*., (2008), in which they studied the toxic effects of aflatoxin B1 on growth performance in ducks.

Dersjant-Li *et al*. (2003) concluded in their review that each mg of AFB1 /kg diet would decrease the growth performance of broiler by 5%. Raju and Devegowda (2000) noted 21% decrease in final body weight at 35 days of age in broiler fed on 0.3 mg AFB, / kg diet, agreeable with the author finding. Zhao *et al*. (2010) noted that at 1 mg/ kg diet of aflatoxin B1, 10% reduction in weight gain at 21 days was reported is agreeable with the present study. Denli *et al*. (2009) reported 15% reduction in weight gain at 42 days of exposure of AFB1 agreeable with the present study. Reduced growth rate may be the only clue for chronic aflatoxicosis and other mycotoxicoses (Raisbeck *et al*. 1991). The reduced growth rate may be related to the disturbances in protein, carbohydrate and lipid metabolism (Cheeke and Shull, 1985). A linear dose dependent decrease in feed intake and body weight gain observed in the aflatoxin B1 treated quail chicks in the present study. Similar studies conducted by Verma *et al*. (2004) have shown that dietary AFB1 adversely affect the growth of birds in a dose depended manner. Feeding contaminated diet with Aflatoxin B1 depressed growth and feed intake of quails as compared to the control groups. These results were agreeable with Cravens *et al*., (2013) who reported that BW gain and FI decreased when levels of 0.75 to 1.5 mg of AFB1/kg of feed were provided to broilers. There is a general agreement that dietary aflatoxin B1 reduces weight gain, feed intake and feed conversion ratio.

The mean antibody titers were significantly lowered in aflatoxin AFB1 treated groups. Comparison within group revealed significant increase (P<0.05) in antibody titre from second week in group B, which is attributed to immune response of body. Same trends were observed in fourth week in group C and D, while group E showed this change in third week due to higher dose of AFB1.Immuno-suppression and lowered immune response to virus vaccine was observed in treated groups with the use of higher levels of Aflatoxin B1 (Table - 4). Guylaine *et al*, 2008 determined the immunotoxicity of aflatoxin B1. The results indicated that dietary exposure of AFB1decreases humoral immunity while inducing an inflammatory response. These impairments in the immune response could participate in failure of vaccination protocols and increased susceptibility to infections in birds exposed to AFB1. Numerous studies performed in different animal species (pigs, rabbits, rats, chicken) exposed to AFB1 failed to observe a significant impairment of the specific humoral response following vaccination or sensitization (Giambrone *et al*., 1985and Marin *et al*., 2002).Tessari *et al*., 2006 reported reduced geometrical mean antibody titres in birds fed on AFB1 and FB_1/kg are in agreement with the present findings. Shivachandra *et al*. (2003) reported significant decrease in HI antibody titer in NDV vaccinated birds. Oguz *et al*. (2003) reported that ND titres were significantly lower ($p < 0.05$) in 100 ppb AF fed chicks, while no significant differences were seen in 50 ppb AF group compared to controls ($p > 0.05$).

Results of hematology revealed that hemoglobin content (g/dl), packed cell volume (%) were significantly decreased in treated quails till the sixth weeks. The groups D and E showed highly significant decrease (P<0.05) in hemoglobin content and PCV as compared to other groups (Figures- 1 & 2). This reduction is linked to reduced feed intake, especially reduced protein intake, malnutrition and disturbances in protein metabolism. Aflatoxins inhibit DNA-dependent RNA polymerase and causes impairment of nuclear DNA template function, resulting in general inhibition of protein synthesis (Gelboin *et al.* 1966; Yu, 1977).

Total leukocyte count (x $10^3/mm^3$)of treated groups was significantly decreased (P<0.05) as compared to the control group (Figure- 3).When compared within groups Hb content was decreased significantly (P<0.05) from third week in group B, while similar changes (P<0.05) were observed in other intoxicated groups from fourth week. Hematocrit experienced significant changes (P<0.05) from third week of toxin intake in groups fed with 0.5, 1 and 2mg/kg of AFB1. Total leuckocyte count experienced significant changes during fourth week in group C, D and E, which can be explained as function of body immune response. Whereas the ESR (mm/hr.) was significantly increased as compared the control group (Figure- 4). Erythrocyte sedimentation rate was significantly changed (P<0.05) from fourth week in B and C while similar significant change (P<0.05) was

Table No. 1: Toxicological Effects of Aflatoxin B1 on the Feed Intake by Quails

Groups	Treatments	Weekly mean feed intake (g/bird)					
		1	2	3	4	5	6
A	Basal diet	35.18 ± 0.06^{aA}	59.37 ± 0.27^{aB}	88.54 ± 0.22^{aC}	102.30±1.08^{aD}	109.30±1.49^{aE}	119.80±0.58^{aF}
B	0.25mg/kg feed	35.22 ± 0.05^{aA}	58.95 ± 0.01^{aB}	88.38 ± 0.08^{aC}	102.72±1.15^{aD}	108.86±1.37^{aE}	117.26±1.17^{bF}
C	0. 5mg/kg feed	35.27 ± 0.04^{aA}	57.70 ± 0.29^{bB}	87.90 ± 0.25^{aD}	84.40 ± 0.78^{bD}	71.48 ± 2.04^{bC}	67.38 ± 1.05^{cC}
D	1mg/kg feed	35.17 ± 0.05^{aA}	48.75 ± 0.27^{cBC}	54.66 ± 0.59^{bC}	55.56 ± 0.64^{cC}	53.76 ± 0.49^{cC}	46.64 ± 0.87^{dB}
E	2mg/kg feed	34.98 ± 0.03^{bA}	46.82 ± 0.20^{dC}	47.62 ± 0.12^{cC}	45.28 ± 0.86^{dC}	41.82 ± 0.72^{dBC}	39.54 ± 0.27^{eB}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table 2: Effects of feed containing different levels of Aflatoxin B1 on body weight gain of quails.

Groups	Treatments	Weekly mean body weight gain (g/bird)					
		1	2	3	4	5	6
A	Basal diet	27.26±0.05^{aA}	45.25±0.03^{aB}	50.2±0.05^{aC}	59.4±0.06^{aD}	63.6±0.25^{aE}	63.4±0.10^{aE}
B	0.25mg/kg feed	27.23±0.05^{aA}	45.17±0.04^{aB}	50.2±0.02^{aC}	59.5±0.03^{aD}	63.5±0.15^{aE}	62.3±0.05^{bE}
C	0.5mg/kg feed	27.18±0.37^{aB}	44.05±0.34^{bCD}	47.3±0.11^{bD}	45.5±0.3^{bCD}	35.2±0.1^{bC}	24.38±0.05^{cA}
D	1mg/kg feed	25.76±0.24^{bBC}	34.19±0.04^{cD}	29.5±0.12^{cC}	22.12±0.1^{cB}	25.3±0.09^{cBC}	16.67±0.21^{dA}
E	2mg/kg feed	25.32±0.06^{cC}	29.40±0.40^{dD}	25.5±0.04^{dC}	20.2±0.1^{dB}	18.3±0.1^{dB}	9.54±0.20^{eA}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table No. 3: Toxicological Effects of Aflatoxin B1 on the Feed Conversion Ratio in Quails

Groups	Treatments	Weekly Feed Conversion Ratio					
		1	2	3	4	5	6
A	Basal diet	1.29±0.002^{aA}	1.312±0.01^{aB}	1.766±0.04^{aD}	1.72±0.01^{aCD}	1.718±0.02^{aC}	1.890±0.01^{aE}
B	0.25mg/kg feed	1.29±0.004^{aA}	1.306±0.02^{aB}	1.762±0.02^{aD}	1.73±0.01^{aCD}	1.716±0.02^{aC}	1.884±0.01^{aE}
C	0.5mg/kg feed	1.298±0.004^{aA}	1.310±0.01^{aB}	1.86±0.07^{bC}	1.86±0.01^{bC}	2.032±0.06^{bCD}	2.76±0.03^{bD}
D	1mg/kg feed	1.366±0.013^{bA}	1.426±0.01^{bB}	1.85±0.01^{bC}	2.512±0.02^{cC}	2.120±0.01^{bC}	2.79±0.04^{bD}
E	2mg/kg feed	1.380±0.003^{bA}	1.594± 0.02^{cB}	1.86±0.05^{bC}	2.24±0.03^{dD}	2.28±0.040^{cD}	4.15±0.07 ^{cD}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table No. 4: Toxicological Effects of Aflatoxin B1 on the humoral immune response in Quails

Groups	Treatments	Weekly Antibody Titer (log_2)					
		1	2	3	4	5	6
A	Basal diet	6 ±0.00^{aA}	6.4 ±0.24 ^{aB}	6.8±0.20^{aC}	7.4±0.24^{aD}	7.8 ± 0.20^{aC}	8.0 ± 0.32^{aF}
B	0.25mg/kg feed	5.8±0.20^{aA}	6.2 ± 0.20^{aB}	6.6±0.24^{aBC}	6.8±0.20^{aC}	6.60 ± 0.24^{bBC}	6.4 ± 0.24^{bBC}
C	0. 5mg/kg feed	5.4±0.24^{aB}	5.4 ± 0.24^{bB}	5.4±0.24^{bC}	5.4 ±0.2^{bB}	4.8 ± 0.20^{cA}	4.8 ± 0.20^{cA}
D	1mg/kg feed	4.6±0.24^{bBC}	4.6 ± 0.24^{cBC}	4.6±0.24^{cBC}	5 ±0.32 ^{bcC}	4.40 ± 0.24^{cB}	3.8 ± 0.38^{dA}
E	2mg/kg feed	4.6±0.24^{bD}	4.6 ± 0.24^{cD}	4.2±0.20^{cC}	4.4±0.24^{cCD}	3.60 ± 0.24^{dB}	2.8 ± 0.20^{eA}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table 5: Effects of feed containing different levels of Aflatoxin B_1 on hemoglobin contents of quails.

Groups	Dose of Aflatoxin B_1	Weekly mean hemoglobin contents (g/dl)					
		1	2	3	4	5	6
A	Basal diet	12.81±0.01^{aA}	12.85±0.00^{aB}	12.92±0.01^{aB}	12.90±0.01^{aBC}	12.93±0.01^{aBC}	12.94±0.01^{aC}
B	0.25mg/kg feed	12.82±0.01^{aA}	12.83±0.00^{aA}	12.92±0.01^{aB}	12.90±0.01^{aB}	12.90±0.01^{aB}	12.92±0.01^{aB}
C	0. 5mg/kg feed	12.82±0.16^{aC}	12.84±0.01^{aC}	12.88±0.01^{bC}	12.85±0.01^{bC}	12.64±0.02^{bB}	11.67±0.02^{bA}
D	1mg/kg feed	12.78±0.01^{bC}	12.76±0.01^{bC}	12.72±0.08^{cC}	12.74±0.01^{cC}	12.23±0.17^{cB}	11.31±0.04^{cA}
E	2mg/kg feed	12.76±0.01^{bC}	12.76±0.01^{bC}	12.72±0.01^{cC}	12.73±0.01^{cC}	11.71±0.05^{dB}	10.62±0.03^{dA}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table No. 6: Toxicological Effects of Aflatoxin B_1 on the Packed Cell Volume of Quails

Groups	Dose of Aflatoxin B_1	Weekly mean packed cell volume (%)					
		1	2	3	4	5	6
A	Basal diet	38.516±0.01^{aA}	38.50 ±0.02^{aA}	38.55 ± 0.04^{aB}	38.62 ± 0.08^{aC}	38.64 ± 0.07^{aC}	38.73 ± 0.01^{aD}
B	0.25mg/kg feed	38.50±0.10^{aB}	38.488±0.01^{aA}	38.53 ± 0.06^{aB}	38.61 ± 0.04^{aC}	38.62 ± 0.01^{aC}	38.74 ± 0.07^{aD}
C	0. 5mg/kg feed	38.498±0.01^{aA}	38.494±0.01^{aA}	38.52± 0.09^{abAB}	38.58 ± 0.08^{aB}	38.58 ± 0.01^{aB}	38.58 ± 0.01^{bB}
D	1mg/kg feed	38.498±0.02^{aB}	38.484 ± 0.02^{aB}	38.43 ± 0.0B5^{b}	37.86 ± 0.09^{bA}	38.80 ± 0.02^{bC}	37.39 ± 0.04^{cA}
E	2mg/kg feed	38.492±0.02^{aC}	38.398 ± 0.01^{bC}	37.96 ± 0.06B^{c}	37.56 ± 0.04^{bB}	36.66 ± 0.12^{cA}	36.13 ± 0.04^{dA}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

Table No. 7: Toxicological Effects of Aflatoxin B_1 on the Total Leukocyte Count of Quails

Groups	Dose of Aflatoxin B_1	Weekly Total Leukocyte Count (X $10^3/mm^3$)					
		1	2	3	4	5	6
A	Basal diet	4.55 ± 0.03^{a}	4.56 ± 0.04^{a}	4.56 ± 0.03^{a}	4.57 ± 0.03^{a}	4.56 ± 0.02^{a}	4.57 ± 0.02^{a}
B	0.25mg/kg feed	4.54 ± 0.02^{aB}	4.55 ± 0.02^{aB}	4.55 ± 0.02B^{a}	4.56 ± 0.04^{aB}	4.52 ± 0.07^{aBb}	4.48 ± 0.02^{abA}
C	0. 5mg/kg feed	4.53 ± 0.04^{aC}	4.53± 0.03^{bC}	4.54 ± 0.03^{bC}	4.53 ± 0.03^{bC}	4.46 ± 0.10^{bB}	4.37 ± 0.05^{bcA}
D	1mg/kg feed	4.52 ± 0.06^{aC}	4.52 ± 0.01^{cC}	4.52± 0.07^{cC}	4.48 ± 0.03^{cC}	4.34 ± 0.05^{cB}	4.25 ± 0.08^{cA}
E	2mg/kg feed	4.52 ± 0.06^{aC}	4.51 ± 0.01^{cC}	4.51 ± 0.07^{cC}	4.47 ± 0.01^{cC}	4.22 ± 0.06^{cB}	4.11 ± 0.03^{dA}

Means with superscripts A-F shows significant difference (P≤0.05) in rows whereas a-e shows significant difference (P≤0.05) with in columns.

observed in third week in group D and E which is due to high level of AFB1 fed to these groups. Donmez, N. *et al.* 2012 recorded the decrease in erythrocyte count, leukocyte count, hemoglobin and hematocrit in aflatoxin treated group are in agreement with the findings of the present studies. The hematological parameters were adversely affected by the AFB 1, added at different levels in the feed of quails. The decreased total leukocyte count was observed at high levels of AFB1. Significant decrease in these parameters was also reported in previous studies (Oguz *et al.* 2000) which reflects deleterious effects of AFB 1 on haemopoietic tissue (Mohiuddin *et al.* 1986) and immune systems (Oguz, 1997).Fung and Clark (2004) recorded increase in TLC of turkeys fed on AFB1 which is in disagreement with the present finding. The variation in the findings may be due to the temperature variations, weather changes, dose levels, geographical distribution or age related factors. Reduction in the hematological values has been reported to be attributed to the aflatoxin induced inhibition of the protein synthesis. The reduction in the mean values of haematocrit, Hb and MCH in AF-fed chicks indicate the deleterious effects of AF on haemopoietic tissue. The decreased value of TLC in the present study is in accordance with the studies of Cravens *et al.* 2015. In conclusion, this study clearly revealed that AFB1 in the diet at levels of 0.5 mg/kg resulted in a reduced growth performance, significantly low antibody titer and has adverse effects on haematological parameters.

Table No. 8: Toxicological Effects of Aflatoxin B_1 on the Erythrocyte Sedimentation Rate of Quails

Groups	Dose of Aflatoxin B_1	Weekly ESR (mm/Hr.) 1	2	3	4	5	6
A	Basal diet	3.58 ± 0.03^{a}	3.57 ± 0.03^{a}	3.57 ± 0.03^{a}	3.59 ± 0.04^{a}	3.58 ± 0.03^{a}	3.57 ± 0.03^{a}
B	0.25mg/kg feed	3.57 ± 0.02^{aA}	$3.57 \pm 0.03A^{a}$	3.58 ± 0.03^{aA}	3.64 ± 0.02^{bB}	3.71 ± 0.07^{bC}	3.71 ± 0.01^{bC}
C	0. 5mg/kg feed	3.58 ± 0.04^{aA}	3.57 ± 0.03^{aA}	3.58 ± 0.03^{aA}	3.69 ± 0.012^{cB}	3.72 ± 0.06^{bC}	3.77 ± 0.02^{bC}
D	1mg/kg feed	3.58 ± 0.03^{aA}	3.58 ± 0.05^{aA}	3.65 ± 0.06^{bB}	3.70 ± 0.02^{cC}	3.81 ± 0.02^{bD}	3.95 ± 0.02^{cE}
E	2mg/kg feed	3.57 ± 0.02^{aA}	3.59 ± 0.05^{bA}	3.71 ± 0.02^{cB}	3.81 ± 0.02^{dC}	4.19 ± 0.06^{Cd}	4.52 ± 0.01^{dE}

Means with superscripts A-F shows significant difference ($P \leq 0.05$) in rows whereas a-e shows significant difference ($P \leq 0.05$) with in columns.

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