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Growth performance and hemato-biochemical alterations in induced aflatoxicosis in white pekin ducks (*Anas platyrhynchos domesticus*)

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Abstract

The present study attempts to analyze growth assessment and hemato-biochemical alterations after experimental induction of aflatoxin B₁ in white pekin ducks at neonatal stages through feed upto 8 weeks of age at different dose rate, 6, 12, 24 & 48 ppb. The experiment was carried out for 8 weeks in 120 number of day old white pekin ducklings, dividing randomly into 5 groups with four treatment groups and one control group comprising twenty four birds in each group with 3 replicates. Mean body weight of ducks of all the groups showed no significant variation from 3rd week to 8th week. However, the mean body weight of the ducks decreased numerically as compared to control group of ducks gradually as the dose of toxin in the feed increased. The values of F.C.R varied from 1.09 to 3.30 during the experimental period. F.C.R of ducks was observed to increase numerically as the level of aflatoxin B₁ increased in feed especially in groups fed with 24ppb and 48ppb. Hematological values of hemoglobin, PCV and TLC significantly decreased in Groups fed with 12ppb, 24ppb & 48ppb with significantly lowest in 48 ppb. Values of MCV, MCHC and MCH reduced significantly in all the treatment groups than control without any significant difference between the groups. Enzymes related to liver function like ALT, AST & GGT & kidney function like BUN, Creatinine showing rising trend in higher level of aflatoxin with decreased serum protein indicating liver & kidney dysfunction mostly. Toxic level at 48 ppb of Aflatoxin B₁ in white Pekin duck is confirmed by this study.

Keywords: Aflatoxin B₁, white Pekin ducks, body weight, FCR, hematology, biochemical, liver enzymes

1. Introduction

Aflatoxicosis represents one of the serious diseases in cattle, buffaloes, chicken, ducks and other livestock population. The cause of this disease has been attributed to the ingestion of various feeds contaminated with *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are one of the highly toxic secondary metabolites derived from polyketides produced by fungal species such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* [1] of which aflatoxin B₁ (AFB₁) is most potent. Aflatoxicosis in poultry is characterized by dullness, listlessness, anorexia with lowered growth rate, poor food utilization, decreased weight gain, decreased egg production and increased susceptibility to environmental stress and increased mortality. It is also associated with anemia and mutagenesis. While extensive information is available on the effects of Aflatoxin in other livestock animals, there is less on ducklings, the most sensitive poultry species [2, 3]. Numerous studies indicate that at least 0.5 mg/kg AFB₁ (cultured or purified) could lead to a significant reduction in BWG [4, 5, 6]. Aflatoxin is known to produce hemolytic anemia by decreasing the circulating mature erythrocytes [7]. Body weight is significantly reduced with higher FCR for the ducks fed diet containing AFB₁ [8]. Feeding 50, 150 and 300 ppb aflatoxin in broiler chicken from 0 to 42 days of age revealed hypoproteinemia, hypoalbuminemia, hypoglobulemia, hypoglycemia, increased serum ALT, AST, ALP, GGT, BUN, creatinine and uric acid, hypocalcaemia, hypertriglyceridemia, hypocholesterolemia and decreased HDL and LDL, increased VLDL and TC/HDL [9]. Further, amongst the duck breeds, white pekin, popular sturdy dual purpose breed, happens to be the most susceptible to aflatoxin. The present study attempts to analyze growth assessment and hemato-biochemical alterations after experimental induction of aflatoxin B₁ in white pekin ducks at neonatal stages through feed upto 8 weeks of age at different dose rate, 6, 12, 24 & 48 ppb. As there is veracity of reports of aflatoxin tolerance levels in ducks, the trial will validate the tolerance of aflatoxins level in white pekin ducks on the basis of growth performance and hemato-biochemical alterations.

2. Materials & Methods

2.1. Experiment

The experiment was carried out for 8 weeks during January to March 2018 in 120 number of one day old white pekin ducklings, dividing randomly into 5 groups with four treatment groups and one control group comprising twenty four birds in each group with 3 replicates. Ducklings were reared under standard management system with ad libitum accesses to feed and sufficient water etc. for a period of 8 weeks. Standard duck feed was procured from commercial manufacturer by replacing maize with wheat to make the feed free from aflatoxin. Feed was tested negative for any aflatoxin before feeding to the experimental ducks. Purified aflatoxin B₁ toxin was procured from commercial sources (Himedia) and these toxins added to the feed with different desired proportion at the dose rate of 6ppb, 12ppb, 24 ppb, & 48ppb through premix which were fed to the ducklings in group II, III, IV & V respectively after 3 days and group I as control. All handlings of aflatoxin were done through bio-safety procedures. Experiment was conducted as per Institutional ethical committee procedure.

2.2. Growth parameters

To evaluate the growth performance and feed conversion ratio (FCR), body weight and amount of feed consumption were measured every weekly interval from day old upto 8th weeks in ducklings of different groups. FCR was calculated as per the formula: Total amount of feed consumed in gram/ total weight gain in gram.

2.3 Hematology

For hematological examination, blood collected in EDTA vial on 6th week from 6 birds randomly in each group. Hematology parameters like Hemoglobin (Hb) by acid hematin method, Packed cell volume (PCV) by micro

hematocrit method, Total erythrocytes count (TEC), Total leucocytes count (TLC), Differential leucocytes count, Mean corpuscular volume (MCV) Mean corpuscular hemoglobin concentration (MCHC) Mean corpuscular hemoglobin (MCH) were conducted. Estimation of Hemoglobin was done by Sahli's Acid Hematin Method, PCV by microhematocrit method, TLC & TEC were done by using Nett-Harrick's fluid as diluents and giemsa stain used for DLC.

2.4. Serum biochemistry

For biochemical examination blood collected in clot activator vial on 6th and 8th weeks from 6 birds randomly in each group. Collected blood samples were centrifuged to obtain serum, which were immediately frozen until submitted for serum biochemistry analysis. The Serum samples were subjected for different biochemical tests like Serum total protein, Albumin, Globulin, Albumin/Globulin Ratio, Triglyceride, Blood Urea Nitrogen, Cholesterol, Creatinine, Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase using standard procedures semi-automatic analyzer diagnostic kit Coral clinical system.

2.5. Statistical analysis

The data of different parameters were analyzed through statistical analysis by using the General Linear Model procedures of SAS® to compare between the groups.

3. Results

3.1 Body weight

Mean Body weight on weekly interval of all the groups depicted in Table no.1 and Chart.1 Mean body weight of ducks of all the groups showed no significant variation from 3rd week to 8th week. However, the mean body weight of the ducks decreased numerically as compared to control group of ducks gradually as the dose of toxin in the feed increased.

Table 1: Weekly body weight (Mean) of different group of ducks

	0 Day	1 ST Week	2 ND Week	3 RD Week	4 TH Week	5 TH Week	6 TH Week	7 TH Week	8 TH Week
Group I (Healthy control)	50.33 ^{ab} ±0.76	144.50 ^a ± 3.58	233.33 ^a ±22.75	371.66 ^a ±34.09	598.33 ^a ±57.06	1213.33 ^a ±117.49	1558.33 ^a ±98.67	1765.00 ^a ± 94.78	1908.33 ^a ± 70.68
Group II (6ppb)	51.50 ^{ab} ±0.84	132.50 ^{ab} ± 1.54	223.33 ^{ab} ±18.37	373.33 ^a ±21.85	573.33 ^a ±69.21	1036.67 ^a ± 73.83	1373.33 ^a ± 77.57	1686.67 ^a ± 110.92	1810.83 ^a ± 105.20
Group III (12ppb)	52.33 ^a ±1.14	120.16 ^b ± 5.82	176.66 ^{bc} ±18.70	350.83 ^a ±52.16	538.33 ^a ±89.00	1050.00 ^a ± 127.72	1365.00 ^a ±110.92	1692.00 ^a ±174.11	1773.33 ^a ±179.62
Group IV (24ppb)	49.16 ^b ±0.54	119.00 ^b ± 7.62	168.33 ^c ±13.01	290.00 ^a ±25.81	485.00 ^a ±37.92	1020.00 ^a ± 90.33	1298.33 ^a ± 80.63	1553.33 ^a ± 125.92	1760.00 ^a ±148.23
Group V (48ppb)	51.66 ^a ±0.66	130.83 ^{ab} ± 1.92	178.33 ^{bc} ± 8.72	316.66 ^a ±42.94	470.00 ^a ±60.60	943.33 ^a ± 113.53	1290.00 ^a ±104.40	1531.67 ^a ± 79.76	1631.67 ^a ±86.38

Means with same superscripts do not differ significantly from each other. (p<0.05)

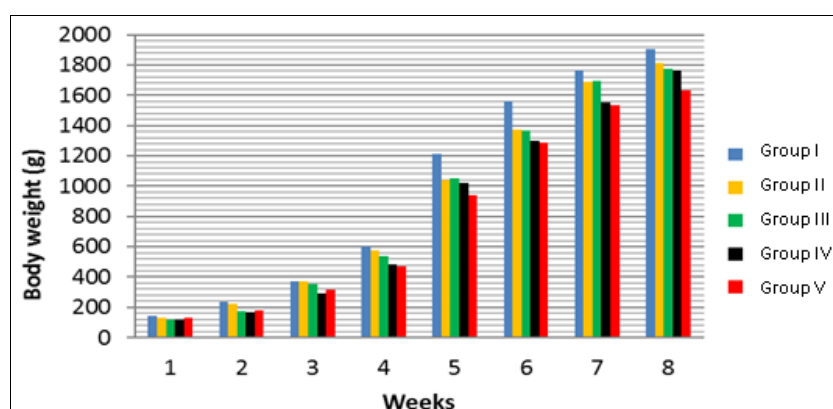


Chart 1: Mean weekly body weight of ducks of different experimental groups

3.2 Feed conversion ratio

Similarly, F.C.R. (Feed Conversion Ratio) was calculated for all the groups using weekly feed consumed and weekly body weight gain and is depicted in Chart.2. The values of F.C.R

varied from 1.09 to 3.30 during the experimental period. F.C.R of ducks were observed to increase as the level of aflatoxin B₁ increased in feed especially in group IV (24ppb) and V (48ppb) when compared to the values of group I.

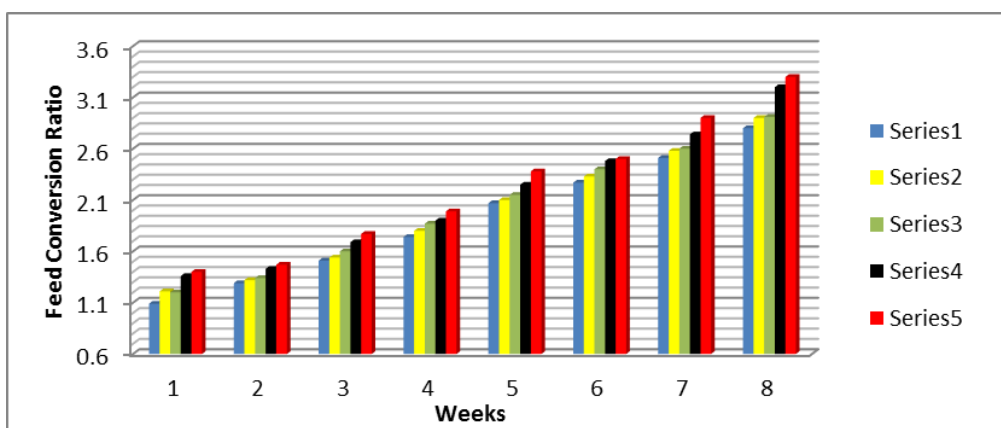


Chart 2: F.C.R of ducks of different experimental groups at weekly interval

3.3. Hematology

Hematological results at 6th week are presented in Table No.2. There was a gradual dose dependent reduction in Hemoglobin and PCV values with significantly lowest in Group III (12ppb), IV (24ppb) & V (48ppb). There was reduction of

Total leucocytes count TLC values in all the treatment groups than control with significantly lowest in 48 ppb. Values of MCV, MCHC and MCH reduced significantly in all the treatment groups than control without any significant difference between the groups.

Table 2: Effect of AFB₁ on hematological parameters of white pekin duck at 6thweek

Hematological Parameter	Group I (Healthy Control)	Group II (6ppb)	Group III (12ppb)	Group IV (24ppb)	Group V (48ppb)
Hb(g/dl)	13.316 ^a ± 0.446	11.1 ^b ± 0.321	9.85 ^b ± 0.3480	9.7166 ^c ± 0.699	9.6333 ^c ± 0.271
PCV (%)	36.8 ^a ± 1.748	33.316 ^{ab} ± 0.862	30.033 ^b ± 0.878	29.45 ^b ± 1.943	29.866 ^b ± 0.918
TEC(10 ⁶ µl)	2.683 ^b ± 0.107	3.3666 ^a ± 0.308	3.4 ^a ± 0.1966	3.25 ^{ab} ± 0.1384	3.3833 ^a ± 0.1621
TLC(10 ⁴ µl)	13.233 ^a ± 0.300	9.8833 ^{cd} ± 0.370	10.55 ^{bc} ± 0.398	10.9333 ^b ± 0.298	9.25 ^d ± 0.1945
MCV(fl)	138.49 ^a ± 9.070	101.554 ^b ± 5.791	90.458 ^b ± 7.385	90.9871 ^b ± 6.044	89.771 ^b ± 6.641
MCHC(g/dl)	36.479 ^a ± 1.639	33.307 ^b ± 0.2683	32.772 ^b ± 0.320	32.9498 ^b ± 0.435	32.2707 ^b ± 0.276
MCH(pg)	49.931 ^a ± 2.117	33.8209 ^b ± 1.935	29.704 ^b ± 2.574	30.0373 ^b ± 2.229	28.907 ^b ± 1.962

3.4 Biochemical alterations

Biochemical analysis results at 6th week depicted in Table No.3 and at 8th week depicted in Table No.4.

At 6th week there was a gradual reduction of Total Protein from group I (Control group) to II (6ppb) & III (12ppb) followed by group IV (24ppb) & V (48ppb). Triglycerides value showed a significant increase in group IV (24ppb) & V (48ppb) than group I, II & III. There was a gradual significant increase value of Blood Urea Nitrogen from group I (control) to group V (48ppb) with a significant difference between the groups. AST & Alkaline phosphatase value revealed highest in group V (48ppb) than control and other treatment groups. ALT showed significant highest value in both group IV & V (24 & 48ppb) than group I, II & III. There was gradual significant dose dependent increase in GGT value from Group

I (Control) to Group V (48ppb).

At 8th week there was a gradual reduction of Total Protein from group I (control group) to II (6ppb) & III (12ppb) followed by group IV (24ppb) & V (48ppb). Albumin & Globulin level revealed similar reduction. Triglycerides value showed a significant increase in group III, IV & V than group I, II. There was a gradual significant increase value of Blood Urea Nitrogen from group I (Control) to group V (48ppb). Creatinine revealed significantly highest value in group IV & V (24 & 48ppb) than group I, II & III. AST value revealed a gradual increased from group II & III (6 & 12 ppb) to highest value in group IV & V (24 & 48ppb). ALT, ALP & GGT values revealed a significant increase in group V (48ppb) than other groups.

Table 3: Effect of Aflatoxin B₁ on Biochemical Parameters of white pekin duck at 6thweek

Biochemical Parameter	Group I(Healthy Control)	Group II (6ppb)	Group III (12ppb)	Group IV (24ppb)	Group V (48ppb)
Total Protein (g/dl)	8.58 ^a ± 0.149	7.06 ^b ± 0.312	7.277 ^b ± 0.1073	5.6025 ^c ± 0.214	5.5075 ^c ± 0.532
Albumin g/dl	2.255 ^a ± 0.22	1.9825 ^a ± 0.029	2.312 ^a ± 0.0764	1.8525 ^a ± 0.194	2.142 ^a ± 0.2015
Globulin g/dl	6.327 ^a ± 0.134	5.0775 ^b ± 0.332	4.965 ^b ± 0.135	3.75 ^c ± 0.316	3.365 ^c ± 0.7118
Alb/Globulin Ratio	0.3587 ^a ± 0.044	0.39607 ^a ± 0.286	0.46766 ^a ± 0.0261	0.5146 ^a ± 0.091	0.873 ^a ± 0.3847
Triglyceride mg/dl	196.44 ^c ± 8.56	217.157 ^c ± 2.78	226.195 ^c ± 18.320	284.945 ^b ± 10.29	338.625 ^a ± 17.983
Blood Urea Nitrogen mg/dl	3.02 ^d ± 0.27	3.35 ^{cd} ± 0.194	3.67 ^c ± 0.096	4.3975 ^b ± 0.1797	5.4025 ^a ± 0.2003
Cholesterol mg/dl	185.74 ^c ± 6.45	147.72 ^d ± 5.321	160.74 ^d ± 3.284	210.845 ^b ± 5.477	274.117 ^a ± 5.0079

Creatinine mg/dl	0.215 ^{ab} ± 0.006	0.1957 ^{bc} ± 0.0132	0.1777 ^c ± 0.012	0.2062 ^{abc} ± 0.0023	0.23225 ^a ± 0.0100
AST (IU/L)	65.2 ^b ± 1.559	66.255 ^b ± 1.307	71.035 ^b ± 3.821	71.1875 ^b ± 3.154	82.07 ^a ± 2.5124
ALT(IU/L)	23.77 ^c ± 0.289	23.162 ^c ± 0.116	23.41 ^c ± 0.0958	24.61 ^b ± 0.210	26.012 ^a ± 0.285
Alkaline phosphatase (IU/L)	339.36 ^b ± 26.08	322.007 ^b ± 7.671	332.81 ^b ± 17.262	373.44 ^b ± 6.0497	426.93 ^a ± 21.92
GGT (IU/L)	2.42 ^c ± 0.147	2.725 ^{bc} ± 0.092	2.7425 ^{bc} ± 0.214	3.08 ^{ab} ± 0.0414	3.33 ^a ± 0.2102

Table 4: Effect of AFB₁ on Biochemical Parameters of white pekin duck at 8th week

Biochemical Parameter	Group I (Healthy Control)	Group II (6ppb)	Group III (12ppb)	Group IV (24ppb)	Group V (48ppb)
Total Protein (g/dl)	8.515 ^a ± 0.3067	7.5275 ^b ± 0.2210	7.365 ^b ± 0.2038	6.4125 ^c ± 0.228	5.9125 ^c ± 0.280
Albumin g/dl	2.3975 ^a ± 0.1836	1.9875 ^{ab} ± 0.4385	1.935 ^b ± 0.1031	1.605 ^{bc} ± 0.1169	1.335 ^c ± 0.1834
Globulin g/dl	6.1175 ^a ± 0.3005	5.54 ^{ab} ± 0.2067	5.43 ^{abc} ± 0.2062	4.8075 ^{bc} ± 0.3119	4.5775 ^c ± 0.450
Albumin/Globulin Ratio	0.3958 ^a ± 0.0401	0.3601 ^a ± 0.0142	0.3583 ^a ± 0.0258	0.3421 ^a ± 0.0452	0.3165 ^a ± 0.0856
Triglyceride mg/dl	239.28 ^a ± 5.660	240.782 ^a ± 7.323	264.505 ^b ± 15.607	329.7 ^b ± 21.455	363.976 ^b ± 12.217
Blood Urea Nitrogen mg/dl	2.6375 ^c ± 0.1261	3.085 ^{bc} ± 0.221	3.0275 ^{bc} ± 0.0739	3.735 ^b ± 0.554	4.82 ^a ± 0.3391
Cholesterol mg/dl	216.68 ^c ± 10.9921	269.96 ^{bc} ± 19.341	282.117 ^{bc} ± 18.154	300.997 ^b ± 24.12	386.282 ^a ± 35.31
Creatinine mg/dl	0.224 ^b ± 0.0132	0.21675 ^b ± 0.0063	0.2925 ^{ab} ± 0.0193	0.316 ^a ± 0.0322	0.3337 ^a ± 0.039
AST (IU/L)	85.457 ^c ± 1.4832	100.58 ^b ± 4.804	105.182 ^{ab} ± 2.999	109.827 ^a ± 2.974	111.882 ^a ± 1.7074
ALT(IU/L)	34.28 ^c ± 1.259	39.282 ^{bc} ± 2.065	42.505 ^{ab} ± 1.538	41.95 ^{ab} ± 2.198	45.725 ^a ± 2.605
Alkaline phosphatase (IU/L)	236.677 ^b ± 22.347	220.265 ^b ± 20.056	274.75 ^{ab} ± 16.674	270.48 ^{ab} ± 9.880	309.69 ^a ± 19.857
GGT (IU/L)	2.7125 ^c ± 0.1784	2.975 ^{bc} ± 0.0462	2.952 ^{bc} ± 0.2092	3.335 ^{ab} ± 0.0290	3.465 ^a ± 0.2027

4. Discussion

There was a gradual dose dependent reduction in mean body weight of ducks of all the treatment groups of 6ppb to 48ppb of aflatoxin B₁ at 3rd week to 8th week. F.C.R of ducks was observed to increase as the level of aflatoxin B₁ increased in feed especially in groups fed with 24ppb and 48ppb when compared with control group. Body weight was significantly reduced for the ducks fed diet containing aflatoxin B₁ with higher FCR [8]. When broilers were fed upto 2.8 mg of AFB₁/kg, there was a negative effect on all performance parameters [10].

Hematological values of hemoglobin, PCV and TLC significantly decreased in group III (12ppb), IV(24ppb) & V(48ppb) with significantly lowest in 48 ppb. Values of MCV, MCHC and MCH reduced significantly in all the treatment groups than control without any significant difference between the groups. In a study, chicks fed with 0.625, 1.25, 2.5, 5.0 and 10.0 ppm aflatoxin for 21 days showed reduction in PCV, Hb and TEC and aflatoxin caused a hemolytic anemia rather than hemorrhagic or aplastic anemia [7]. Significant reduction in PCV and Hb levels in broilers fed 1 ppm aflatoxin from 0 to 4 weeks of age was observed [11]. Reduced hematological values due to experimental aflatoxicosis in chickens are reported by various workers [12, 13, 14, 15]. Almost similar trends of biochemical alterations were observed at 6th and 8th week. There was a gradual dose dependent reduction of total protein in ducks fed with 6ppb to 48ppb of AFB₁. Globulin level revealed similar reduction. Triglycerides value showed a significant increase in group IV (24ppb) & V (48ppb). AST & Alkaline phosphatase value revealed highest in group V fed with 48 ppb than control and other treatment groups. ALT showed significant highest value in both group IV (24ppb) & V (48ppb) than group with lower doses. There was gradual significant dose dependent increase in GGT value from group I to group V. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are serum enzymes and the increased activity of these enzymes can be used as a tool to indicate abnormal liver activities caused by AFB₁ [8]. Alterations in serum levels of ALT and AST are liver specific and have been considered as a tool for studying varying cell viability and changes in cell membrane permeability [16]. There was a gradual significant increased value of Blood Urea Nitrogen from 6ppb to 48ppb with a significant difference between the

groups. Creatinine revealed significantly highest value in group 24 & 48 ppb at 8th week. Based on the increase in serum urea N levels, it is likely that impaired protein synthesis was due to lower utilization of amino acids [17]. Biochemical alterations are indicative of liver and kidney damage at the dose rate of 24 ppb and 48 ppb of aflatoxin B₁ in this study. Decrease in serum protein due to experimental aflatoxicosis reported by various workers [18, 19, 20, 21, 22]. Feeding 50,150 and 300 ppb aflatoxin in broiler chicken from 0 to 42 days of age revealed hypoproteinemia, hypoalbuminemia, hypoglobulinemia, hypoglycemia, increased serum ALT, AST, ALP, GGT, BUN, creatinine and uric acid, hypocalcemia, hypertriglyceridemia, hypocholesterolemia and decreased HDL and LDL, increased VLDL and TC/HDL. The consumption of feed contaminated with AFB₁ can affect digestive enzyme activities, nutrient digestibility and utilization, leading to poor animal performance [9]. In a study, a significant decrease in serum protein, albumin, albumin-globulin ratio, glucose, calcium, phosphorus, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and a significant increase in ALT, AST, ALP, BUN, creatinine, uric acid and very low density lipoprotein (VLDL) in layer chicks fed 0.5 ppm AF for 12 weeks was observed [21].

5. Conclusion

Toxic level at 48 ppb of aflatoxin B₁ in white pekin duck was confirmed by this study with reduced growth and higher FCR at 8th week. Hemoglobin, PCV, TEC was lowest in 48 ppb indicating adverse effect on erythrocytes. Enzymes related to liver function like ALT, AST & GGT & kidney function like BUN, Creatinine showing rising trend in higher level of aflatoxin with decreased serum protein indicating liver & kidney dysfunction mostly.

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