

Pathological Effects of Graded Doses of Aflatoxin B1 on the Development of Testes in Juvenile White Leghorn Males

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Abstract

Current experiment was planned to investigate the deleterious effects of the graded dose of aflatoxin B1 (AFB1) on White Leghorn Male Birds. For this purpose, 100 birds of 8 week age were divided into 4 equal groups and reared on feed contaminated with different doses of AFB1 for 10 weeks. Group A was kept as a control group and was fed with normal toxin free diet, group B, C, and D were offered feed containing 100 ppb, 200 ppb, and 400 ppb of AFB1 respectively. The birds were euthanized at the 4th and 10th week of the experiment. Clinical signs, behavioral changes, absolute and relative organ weight of testes, sperm motility were measured. Cellular immune response was observed through CCA, P-HAP, and antibody response against SRBC. Results showed a dose-dependent decline in the immune response of birds with the increase in the level of AFB1 in the feed. A significant decrease in the serum levels of testosterone, prolactin, and LH was observed. Grossly, testicular size and volume were reduced in AFB1 fed birds while histological examination showed mild to moderate and severe necrosis of testicular parenchyma, with partial to complete arrest of spermatogenesis. Very few spermatozoa were found in group C while they were almost absent in group D which was offered a diet containing 400 ppb AFB1. The above-mentioned results showed that AFB1 had severe toxic effects on the reproductive and immunological parameters of WLH birds in a dose-dependent manner.

Introduction

Poultry industry is a vibrant source of a balanced diet for poor masses of developing countries. It is a cheap source of quality protein for low-income people in Pakistan. Many people are earning their livelihood by working in the poultry industry directly or indirectly. However, one of the obstacles in the growth and development of the poultry industry is the contamination of mycotoxins and pesticides contamination in poultry feed (Gul *et al.*, 2020)

This fungal contamination poses a serious threat to crops in the form of secondary metabolites which are called mycotoxins (Moussa *et al.*, 2020). Some of these are known to humans from ancient times while others are being recently discovered. There are different types of mycotoxins like aflatoxins, ochratoxins, zearalenone, fumonisins, T-2 toxins, DON, and DAS etc. (Saleemi *et al.*, 2017; Imran *et al.*, 2020; Gohar *et al.*, 2020).

Aflatoxins are the metabolites produced from the *Aspergillus* sp. mainly *A flavus* & *A parasiticus* and further classified as Aflatoxin B1, B2, G1, G2, M1 & M2 (Usman *et al.*, 2019; Sana *et al.*, 2019). Aflatoxin B1 was discovered as the main cause of the death of the young turkeys in England in the 1960s in the most notorious disease known as Turkeys X Disease (Kim *et al.*, 2013). AFB1 is a carcinogenic metabolite of aflatoxin but it is present in an inactivated form which gets activated by the hepatic microsomal cytochrome P450 enzyme. According to the report of (IARC) AFB1 & AFB2 are highly carcinogenic to humans (Rawal *et al.*, 2010; Mahrous *et al.*, 2020).

Aflatoxins lead to a decline in daily weight gain, egg production, decreased levels of serum proteins, and poor feed conversion ratio in poultry birds in Pakistan (Saleemi *et al.*, 2017; Naseem *et al.*, 2018). Liver is the principal target organ for aflatoxins followed by kidneys, lungs, thymus and bursa (Manafi *et al.*, 2014). Aflatoxins compromise the fertility of birds by affecting the reproductive system in several ways e.g. it can prolong the time to attain sexual maturity by male birds. Feeding goats with aflatoxin contaminate feed for a longer period resulted in testicular degeneration (Maryamma & Sivades 1975). There was a decrease in testicular size and testosterone concentration in blood plasma because of aflatoxicosis. AFB1 causes a decrease in the number of Leydig cells, spermatids, and spermatocytes. Giant, immature and multinucleated spermatids are also produced as a result of aflatoxicosis (Faridha *et al.*, 2007; Juman *et al.*, 2020)

AFB1 not only causes immunosuppression but also drastically changes the hematological parameters (Hb, PCV, TEC & TLC) and it also affects the serum biochemistry (Afzal and Saleem, 2004). Gross lesions seen on postmortem of the AFB1 affected animals and birds showed prominent enlarged pale-colored liver and distended ball bladder due to poor feed intake (Kumar and Balachandran, 2009). Many enzymes present in the blood circulatory system are severely affected due to aflatoxicosis leading to poor digestion of many useful ingredients essential for optimal performance of the body. As a consequence of which birds show poor body condition and poor body weight (Carrillo *et al.*, 1982). Aflatoxins not only lower the number of circulating WBC but also reduces their phagocytic efficiency as a result of which the bird's immune system gets compromised and the birds become susceptible to many diseases (Kubena *et al.*, 2001; Abdel-Sattar *et al.*, 2019)

In poultry, male infertility is the major problem especially at breeder flocks where we have to do artificial insemination because of decreased male libido or infertility. Aflatoxins may be one of the major causes of this reproductive problem. Therefore, the current study was designed to examine;

- a. Adverse effects of graded doses of aflatoxin B1 on the development of testes in juvenile white leghorn males.
- b. Gross and microscopic changes induced by aflatoxin B1 at different doses in the male reproductive system of birds.

Materials And Methods

Experimental design

In the current trial, 100 commercial White Leghorn male cockerels of 8 weeks age were procured from a commercial layer farm. Birds were allowed to acclimatize for 2-3 days at experimental sheds of the Department of Pathology, UAF. Birds were randomly divided into four equal groups namely groups A, B, C, and D. The time frame of the trial was 10 weeks. There were four groups of birds along different treatments comprising A (control), B (AFB1 100 ppb), C (AFB1 200 ppb), and D (AFB1 400 ppb). Feed free from toxin binders were prepare and then known quantity of AFB1 was added to it after experimental

production on rice substrate. Pure *A. flavus* cultures, link: Fries. A (CECT 2687) and *A. parasiticus* cultures obtained from Culture Collection Center University De Valencia, Spain was maintained and inoculated on Sterilized Potato dextrose agar and left for incubation for 7 days at 27°C. These cultures were used for production aflatoxins by inoculating on rice by the method of Shotwell *et al.* (1966) & modified by Hussain *et al.* (2008).

Parameters: The clinical signs and behavioral alterations were observed twice daily and got weekly score of the birds. Birds were humanely euthanized twice, first on the 30th day of the trial while the second on the final day of trial. Four birds from every group were selected on random bases which were slaughtered on the day of each slaughtering. At each killing testes weight and volume were noted and relative weight was calculated as % of body weight.

Relative Organ Weight (%) = (Abs. organ weight (g)/Bodyweight (g)) X100.

Male Reproductive system Parameters: Semen was collected by massaging the abdominal area as described by Burrows and Quinn (1937). Semen was collected twice a week. Motility was assessed by sperm motility Computerized Automatic Sperm Analyzer (CASA). Briefly, a small drop of semen of 5µL was placed on a microscopic slide and observed under CASA. Hormonal profile of serum testosterone, prolactin and luteinizing hormone was measured by radioimmunoassay using commercially available kits following all the manufacturer instructions. For testosterone TESTO-CT2 kit of Cisbio Bioassays France, for prolactin RF02N RIAKEY Prolactin IRMA Tube II and for luteinizing hormone RF03N, RIAKEY LH IRMA Tube II kits Shin Jin Medics Inc. Korea were used.

Immunological Parameters

Antibody Titers against Sheep Red Blood Cells (SRBCs): SRBCs were injected by the intravenous route in 3 birds from each group on the 14th day of the experiment. A booster dose was injected 7 days after the initial administration of SRBCs. To check the immune response against the foreign SRBCs blood was collected on the 7th day after the primary dose and 7 days after the booster dose administration (Delhanty and Solomon, 1966).

Lymphoproliferative response against Avian tuberculin: Avian tuberculin was injected into inter digital space between the 3rd and 4th digit in 3 birds from each group on the 40th day of the experiment. The thickness of inter digital space was measured after 24 and 48 hours after the initial administration of PHA-P (Corrier, 1990).

Carbon Clearance assay (CCA) (Activity of Mononuclear phagocytic system): Carbon clearance assay was assessed by using the method of Sarker *et al.* (2000). The chicken circulatory macrophages phagocytic capability was determined. At 3000g for 30 minutes the Pelikan 4001 ink was centrifuged for the collection of supernatant fractions and 1 ml/kg of body mass, the ink was infused into the wing vein (right-side) to six birds from each group. Before injection means at 0 minute and 3 minute and 15 minutes of after injection blood from wing vein (left side) was collected (200 µl/chick). Immediately

collected blood was transferred into a tube having 4 ml of 1% sodium citrate. Collected blood was centrifuged at 500 g for 4 min. At 640 nm wavelength, the OD value of supernatant was measured. The overall measure of non-phagocytized carbon units staying in the supernatant from each wing of birds was done by deducting the optical density (OD) value at three and fifteen minutes as of that of zero minutes.

Absorbance (%) = (Abs. of a specific time- Abs.at time 0 mint)/(Absorbance at time 0)×100

Gross and microscopic pathology of Testes

The testicular tissues of the birds that died during the experiment or were euthanized, were observed for gross lesions. The tissues including testes were preserved in 10% neutral buffered formalin for histopathology (Bancroft and Gammable, 2008).

Statistical Analysis:

The data obtained from experiment was analyzed statistically by analysis of variance (ANOVA) and groups means were compared by Duncan's multiple range test (DMR-Test) by using M-Stat c statistical software.

Results

Clinical Signs and Behavioral Alterations

Clinical signs including alertness of the birds to signal, activity of the birds, crowing behavior which is that the bird is getting mature was noted twice daily. Only the control group showed good signs of maturity while the other birds showed late signs of maturity in a dose gradient manner. The least clinical signs were showed by group D which was fed AFB1 400 ppb (Table 1).

Absolute organ weights

Testes weight was significantly lower in group D as compared to other groups as in Table 2. Groups A, B and C showed non-significant difference with each other. In testes weight of all four groups followed the trend as A> B > C > D. Testes volume was significantly higher in control group A among other groups. In testes volume of all four groups followed the trend as A> B > C > D. Group D has the least testicular volume showing severing testicular atrophy by the AFB1 400ppb fed to the birds in that group.

In 2nd killing testes weight was significantly higher in control group A. Group D testes weight was significantly lower as compared to control group. Testes volume group A was significantly higher compared to group D while non-significant with group B and C.

Relative organ weight of WLH male layers

In the 1st killing the relative organs weight of testes of group D was significantly lower as compared to control group A. The trend seen in testes weight were as follows ($A > B > C > D$). In second killing there was no significant change in the relative weight of the testes in all four groups however control group has maximum relative testes weight and group D has least relative testes weight. There was no significant change in the relative volume of the testes in all four groups however control group has maximum relative testes volume and group D has least relative testes volume Table 3.

Antibody titers against SRBC

Titers at 7th Day of Primary Dose

Antibody titer of total antibodies at the 7th day of the initial dose of group A and group B was non-significant with each other and significantly higher as compared to groups C and D while the group D showed the lowest antibody titer on the 7th day. The IgM titers were non-significant among all groups but group D showed the least value of IgM and group A showed the maximum value of IgM on 7th day. The IgG titers of group B were significantly higher as compared to groups C and D while group D showed the least antibody titer for IgG (Figure a1).

Total Antibody titers on the 14th day of group A were significantly higher as compared to group C while groups C and D were significantly lower as compared to the control group. The IgM titer of group A was significantly higher as compared to group C. The IgG titers were significantly higher in group A as compared to groups C and D. groups A and B were non-significant with each other while group D showed the least value. Antibody titers at the 7th day of a booster dose of group A were significantly higher as compared to groups B, C and D. While the group D showed the minimum value. The IgM titer of group A was significantly higher as compared to other groups. The IgG titers of group D were significantly lower as compared to control group A.

On the 14th day of a booster dose, the total antibody titers of group A were significantly higher than all other groups with group D showing the minimum levels of total antibodies. The IgM titers were non-significant in all groups but group A showed the maximum titer for IgM as compared to other groups. The IgG titer of group A was significantly higher as compared to other groups as shown in figure b1.

Mononuclear Phagocytic Response Carbon Clearance Assay

In-vivo phagocytic response of mononuclear cells to carbon particles at 3 minutes response groups had non-significant difference among all groups. At 15 minutes, group D was having significantly higher absorbance as compared to the control group and all other groups. The control group was significantly lower compared to other groups. All the groups followed a trend such as group ($D > C > B > A$) which means the phagocytic activity in the group D was least to clear the carbon injected into the bloodstream showing a weaker immune response while the lowest absorbance value at response 15 minute shown by control group means that the immune system was functioning very well and responded significantly by

clearing the carbon injected into the body by the phagocytic activity shown by the mononuclear cells (Fig. 2).

Lymphoproliferative response to avian tuberculin

At 24 hours, control group (A) showed a response that was significantly higher as compared to all other groups especially group D. While group D was significantly lower as compared to group B and group B and group C were significantly different from each other at 24 hours response. At response time 48 hours control group A was significantly higher as compared to all groups. Group B and group C were non-significant to each other while both groups showed response significantly higher as compared to group D. At response 72 hours group A, B and C did not show significant difference with each other, however, the lymphoproliferative response of group D was significantly lesser compared to the control group (Fig. 3).

Sperm Motility Percentage

Sperm motility was decreased in all groups with increasing doses of toxin as shown in Table 4.

Reproductive Hormones

Serum levels of these reproductive hormones (testosterone) clearly follow the trends which are Group A > B > C > D. This means that the level of this hormone decreased as there was an increase in the level of AFB1 in their diet as shown in Table 5. The level of luteinizing hormone did not show any significant increase in its level but the level of prolactin was increased significantly with increase in dose of toxin.

Gross Lesions

There was no obvious gross lesion seen in the control group. Testes showed normal size and weight. However, there was a significant decrease in testicular size in a dose-dependent manner between the three groups fed with AFB1 contaminated feed (Fig. 4).

Testes Histopathology

Seminiferous tubules in control group A showed normal appearance. The lumen of the tubule was patent. Spermatogenesis developmental stages such as spermatogonia, spermatocytes, spermatids and mature spermatozoa were found. The presence of clusters of spermatozoa indicated normal testicular parenchyma (Fig. 5a & b). Testicular parenchyma in group B also showed normal appearing seminiferous tubules. All the morphological stages of spermatogenesis i.e. spermatogonia, spermatids, spermatocytes and spermatozoa were visible. However, in some of the seminiferous tubules partial arrest of spermatogenesis was present (Fig. 5c & d). Likewise, all the stages of spermatogenesis were present in group C. But the number of spermatozoa was less indicating partial arrest of spermatogenesis. In a few places, the lumen of the seminiferous tubule was not patent (Fig. 5c & d). In group D, arrested spermatogenesis was evident in many areas as the lumen of the seminiferous tubules was not patent. Developmental stages of spermatogenesis were seen except the mature spermatozoa. In some tubules,

few spermatozoa were also present. Number of spermatozoa was less; necrotic changes were also present in seminiferous tubule (Fig. 5e & f).

Discussion

The poultry industry is a vital source of balanced diet in poor masses in our country as it is the cheapest source of protein for people. But these days the poultry sector is facing serious issues by an ever-growing threat caused by the toxins produced by fungus species like *A. flavus*. Aflatoxins can cause male infertility in chicken especially at the breeder level which is one of the major threats.

The current experimental study was designed to investigate the pathological effects of graded doses of AFB1 in the male reproductive system of WLH breeder male birds. In group B birds were depressed, with ruffled feathers, showed poor growth, less attraction towards feed and water intake was more that also leads to watery droppings. Similar signs and lesions have been reported by Verma *et al.* (2004) in broilers administered with aflatoxins and ochratoxins. Denil and Okan. (2006) also reported same results as reported by our study. Hussain *et al.* (2008) also reported similar results to ours like poor growth when broiler was exposed to graded doses of aflatoxins. Clinical signs of aflatoxicosis in WLH male breeders observed in the current study were in line with Ortatli and Oguz, 2001; Kubena *et al.*, 1998 and Khan *et al.*, 1994. Similar results were also reported by Yunus *et al.* (2011).

Immunological response on the birds was observed through CCA, P-HAP and antibody response against the SRBC. A regular trend was observed in all three immunological parameters (CCA, P-HAP and antibody response against SRBC) means that there was a gradual decrease in the immune response shown by the groups from A to D. This clearly explained the adverse effect of AFB1 on the immune system of the body of birds. Similarly, results were also observed by

Change in organ size was also observed in both slaughtering at 4th and 8th week of experiment there was increase in the size of liver and kidneys from group A to D while there was gradual decrease in the testicular size and testicular volume with the increase in the dose of the AFB1 from group A to D. similar results to our study were observed by Celyk *et al.* (2003); Hussain *et al.* (2008) and Daniel *et al.* (2009).

Sperm motility also showed a regular trend as it decreased from Group A >B >C >D, showing that as the dose of AFB1 was increased in the feed there was decreased in sperm motility and increase in sperm abnormalities. Similar results were observed by Tajik *et al.* (2007), Clarke *et al.* (1987). Fapohunda *et al.* (2008) also observed the same results when they fed AFB1 contaminated corn feed to the mice. Different reproductive hormones like testosterone, LH and prolactin also followed the same trend. Control group has the highest level of reproductive hormones testosterone, LH and prolactin however, least levels of these hormones were observed in group D. Abdel-Haq *et al.* (2000), Adedara *et al.* (2014), Clarke *et al.* (1987) and Hasanzadeh *et al.* (2011).

Serum level of testosterone, luteinizing hormone and prolactin were decreased in dose dependent manner as compared to control birds and these results are consistent with findings of Ewuola *et al.* (2014) who

reported the reduced level of testosterone in goats. Similar kinds of results were also reported in white male chicken and rats (Bbosa *et al.*, 2013; Hasanzadeh *et al.*, 2011). This reduction in the level of testosterone may be attributed to reduction in the number of leydig cells. There was no significant decrease on level of LH and our findings differ from Hasanzadeh *et al.* (2011) who reported reduction in the level of this hormone.

In control group normal testicular parenchyma was present tubule having patent lumen and normal appearance of seminiferous tubules. Spermatogenesis developmental stages like spermatogonia, spermatocytes, spermatids and mature spermatozoa are found. Testicular parenchyma in group B is indicating normal appearance of seminiferous tubule. All the stages of spermatogenesis are also present. Spermatogonia, spermatids, spermatocytes and spermatozoa all stages are present. However at few places partial arrest of spermatogenesis was present. All the stages of spermatogenesis are present in Group C. Number of spermatozoa were less indicating partial arrest of spermatogenesis. At few places, lumen of seminiferous tubule is not patent. Group D is indicating that at few places seminiferous tubule lumen is not patent. Developmental stages of spermatogenesis are seen except spermatozoa stage. In some tubules, few spermatozoa are also present, otherwise spermatogonia, spermatids are present indicating the arrest of spermatogenesis. Numbers of spermatozoa are less, necrotic changes are also present in seminiferous tubules. Similar results to our were observed by Ahmed *et al.* (2012); Adedara *et al.* (2014); Ewuola *et al.* (2014); Supriya *et al.* (2014); Kourousekos & Theodosiadou, (2015) and Murad *et al.* (2015). The results of the present study concluded that AFB1 intoxication leads to decrease in body weights, feed intake in dose related manner. The gross and microscopic changes in the aflatoxin groups were more pronounced. There was marked decrease in testosterone levels and other hormones in a dose dependant manner from group A to D. similar results were observed by Mukuma *et al.* (2016); Hasanzadeh *et al.* (2011), El-Katcha *et al.* (2017) and Eraslin *et al.* (2006).

The results of the present study concluded that AFB1 intoxication leads to decrease in body weights, feed intake in dose related manner. The gross and microscopic changes in the aflatoxin groups were more pronounced with the increase in the level of the toxin offered in the feed. Decreased in testicular size and volume and reproductive hormones like LH, prolactin and testosterone was evident with increase in dose of AFB1.

Declarations

Ethical approval for the use of experimental birds

The birds in this experimental study were used after the approval of the experimental plan by the Graduate Studies and Research Board, University of Agriculture, Faisalabad (UAF), Pakistan.

Consent to participate; It is declared that all the authors are agreed to participate for editing in this article.

Consent to Publish; It is the collective consent of all the authors for the publication of this data.

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Authors Contribution; Experimental designing and analysis; Anas Ashraf, Muhammad Kashif Saleemi, Mashkooor Mohsin, Shafia Tehseen Gul,

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Conflict of Interest; All the authors declare no conflict for the publication of this article.

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Tables

Table 1: Clinical signs and behavioral alterations of WLH breeder males fed with AFB1 intoxicated feed

Weeks	Behavioral changes shown by birds	Score	Groups			
			Control	AFB1 100 ppb	AFB1 200 ppb	AFB1 400 ppb
1	Alertness of the bird	0-4	4	4	4	4
	Mating behavior	0-4	0	0	0	0
	Crowing	0-4	1	1	1	1
	Foaminess in the droppings	0-4	0	0	0	0
	Total		5	5	5	5
2	Alertness of the bird	0-4	4	4	3	3
	Mating behavior	0-4	0	0	0	0
	Crowing	0-4	2	1	1	1
	Foaminess in the droppings	0-4	1	1	1	0
	Total		7	6	5	4
3	Alertness of the bird	0-4	4	4	3	3
	Mating behavior	0-4	1	0	0	0
	Crowing	0-4	3	2	2	2
	Foaminess in the droppings	0-4	1	1	1	1
	Total		9	7	6	6
4	Alertness of the bird	0-4	4	3	3	3
	Mating behavior	0-4	1	1	1	1
	Crowing	0-4	4	3	2	2
	Foaminess in the droppings	0-4	1	1	1	1
	Total		10	8	7	7
5	Alertness of the bird	0-4	4	3	3	3

	Mating behavior	0-4	2	2	2	1
	Crowing	0-4	4	3	2	2
	Foaminess in the droppings	0-4	2	2	2	2
	Total		12	10	9	8
6	Alertness of the bird	0-4	4	3	3	2
	Mating behavior	0-4	3	2	2	2
	Crowing	0-4	4	3	2	2
	Foaminess in the droppings	0-4	2	2	2	2
	Total		13	10	9	8
	Alertness of the bird	0-4	4	3	3	2
7	Mating behavior	0-4	4.	3.	3	2
	Crowing.	0-4	4.	3.	3	3
	Foaminess in the droppings	0-4	3	2	2	3
	Total		15	11	11	10
Grand total		112	71	57	52	48

Table. 2: Absolute organ weight of WLH male layers fed with different levels of Aflatoxins B1 after 1st slaughtering and 2nd slaughtering (Mean ± SD)

Group	1 st slaughtering		2 nd slaughtering	
	Testes	Testes	Testes	Testes
	Weight	Volume	Weight	Volume
Control	8.525 ± 0.39a	9.250 ± 1.06a	7.350 ± 0.21a	7.175 ± 0.46a
AFB1 100ppb	7.500 ± 0.42a	7.625 ± 0.18ab	6.500 ± 0.00ab	6.500 ± 0.71ab
AFB1 200ppb	7.332 ± 1.18a	6.750 ± 0.35bc	5.105 ± 0.89ab	4.750 ± 0.85b
AFB1 400ppb	4.700 ± 0.36b	5.275 ± 0.32b	4.393 ± 1.57b	4.075 ± 1.45ab

Values in each column followed by different letters are significantly different ($p \leq 0.05$)

Table 3; Relative organ weights of WLH males fed with different levels of Aflatoxins B1 on 1st slaughtering and 2nd slaughtering (Mean ± SD)

Group	1 st slaughtering		2 nd slaughtering	
	Testes	Testes	Testes	Testes
	Weight	Volume	Weight	Volume
Control	0.509 ± 0.01a	0.551 ± 0.03a	0.475 ± 0.11a	0.465 ± 0.12a
AFB1 100ppb	0.470 ± 0.04a	0.477 ± 0.01ab	0.372 ± 0.00a	0.372 ± 0.04a
AFB1 200ppb	0.455 ± 0.09a	0.418 ± 0.03bc	0.317 ± 0.08a	0.295 ± 0.07a
AFB1 400ppb	0.308 ± 0.02b	0.346 ± 0.03c	0.295 ± 0.05a	0.275 ± 0.05a

Values in each column followed by different letters are significantly different ($p \leq 0.05$)

Table 4: Motile Sperms percentage of each ejaculate (Mean ± SD)

Ejaculate	Experimental Groups			
	A	B	C	D
1	59.90±0.26a	58.20±0.21b	49.20±0.18c	39.20±0.65d
2	64.75±0.65a	53.50±0.41b	47.80±0.16c	37.82±0.17d
3	68.75±0.21a	52.50±0.41b	45.60±0.16c	31.65±0.19d

Values (Mean ± SD) in each column followed by different letters are significantly different ($p \leq 0.05$)

Table 5: Levels of Reproductive hormones after Final killing (Mean ± SD)

Groups	Testosterone ng/ml	Prolactin mIU/ml	LH mIU/ml
A	2.484±0.39a	0.065±0.1d	0.014±0.00a
B	2.347±0.52a	0.230±0.6c	0.045±0.06a
C	0.933±0.13b	0.522±0.06b	0.017±0.01a
D	0.618±0.23b	0.775±0.08a	0.014±0.00a

Values (Mean ± SD) in each column followed by different letters are significantly different ($p \leq 0.05$).

Figures

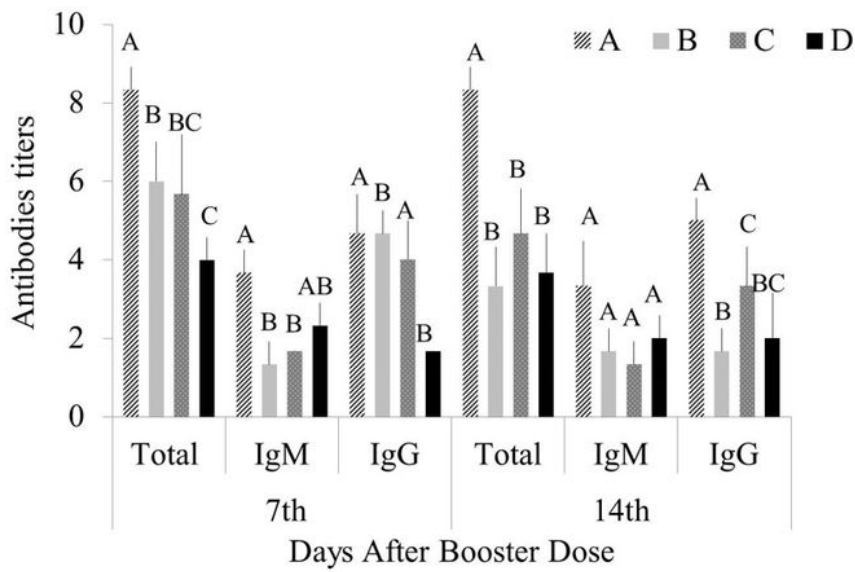
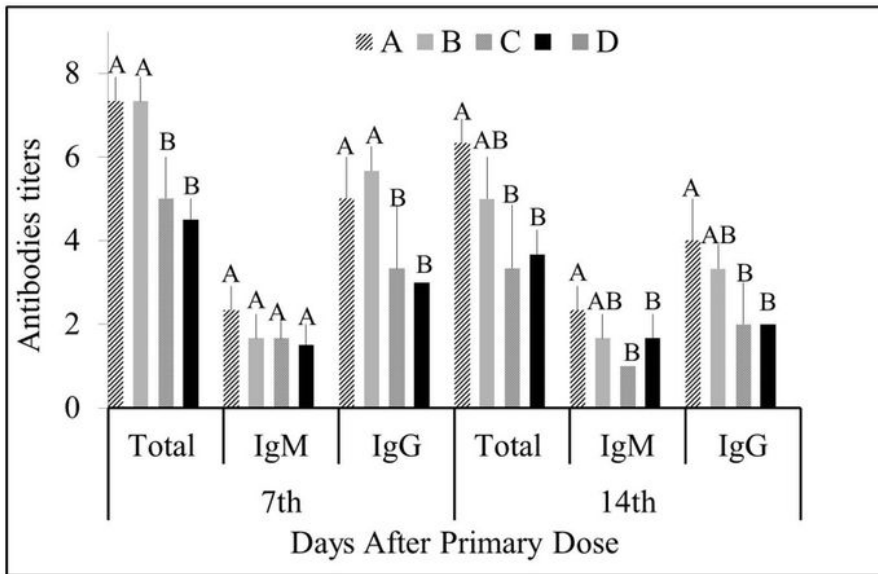


Figure 1

a. 1: Antibodies titers of WLH layer males against sheep RBC's (SRBCs) fed with different levels of AFB1 (Mean ± SD). b. 1: Antibodies titers of WLH layer males against sheep RBC's (SRBCs) fed with different levels of AFB1 (Mean ± SD).

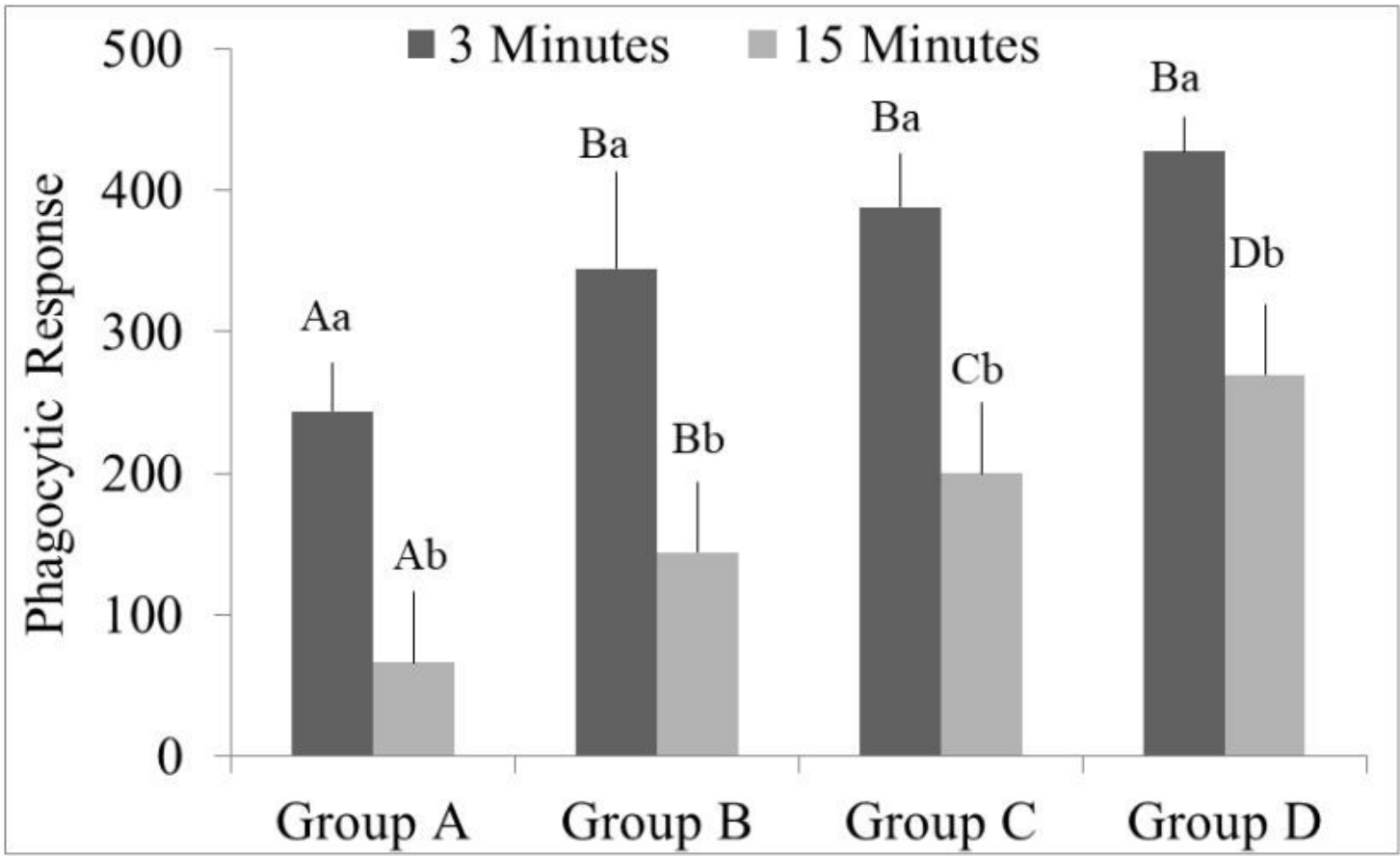


Figure 2

In-vivo phagocytic response of mononuclear. Cells to carbon particles in the WLH breeder males fed AFB1 intoxicated feed.

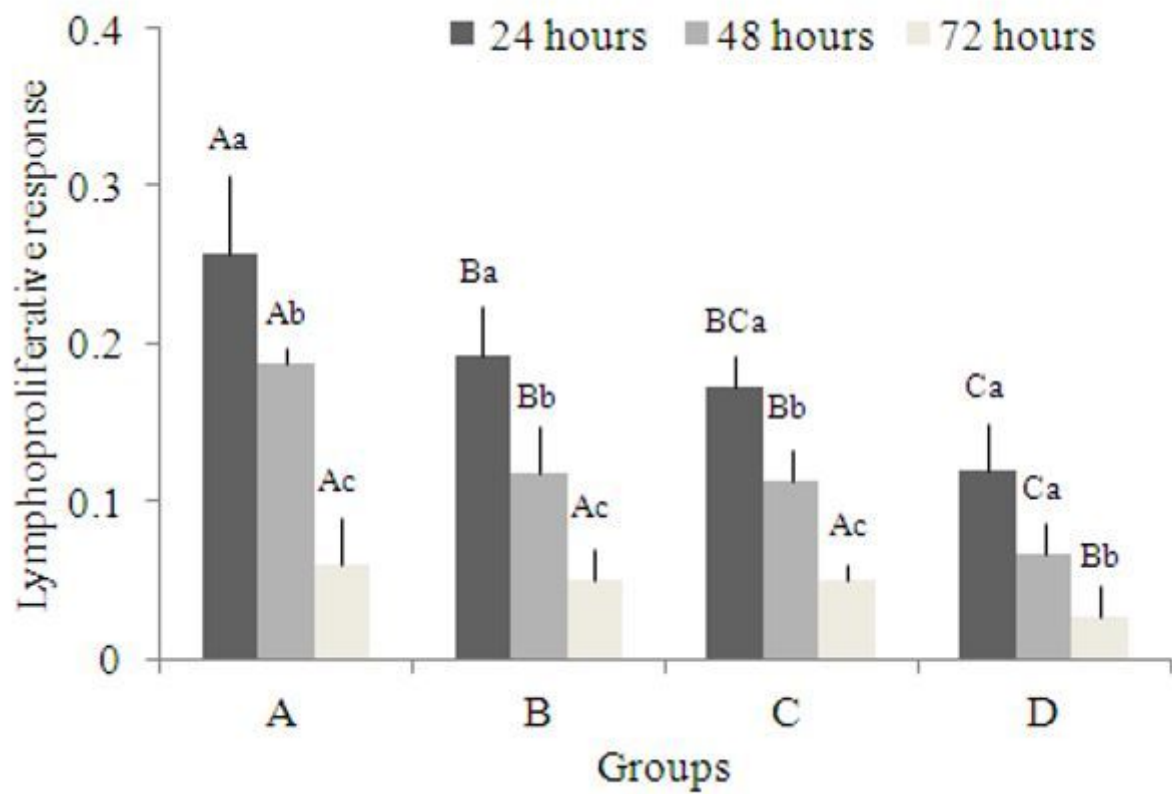


Figure 3

Lymphoproliferative response to avian tuberculin in White Leghorn male layers fed with different levels of AFB1

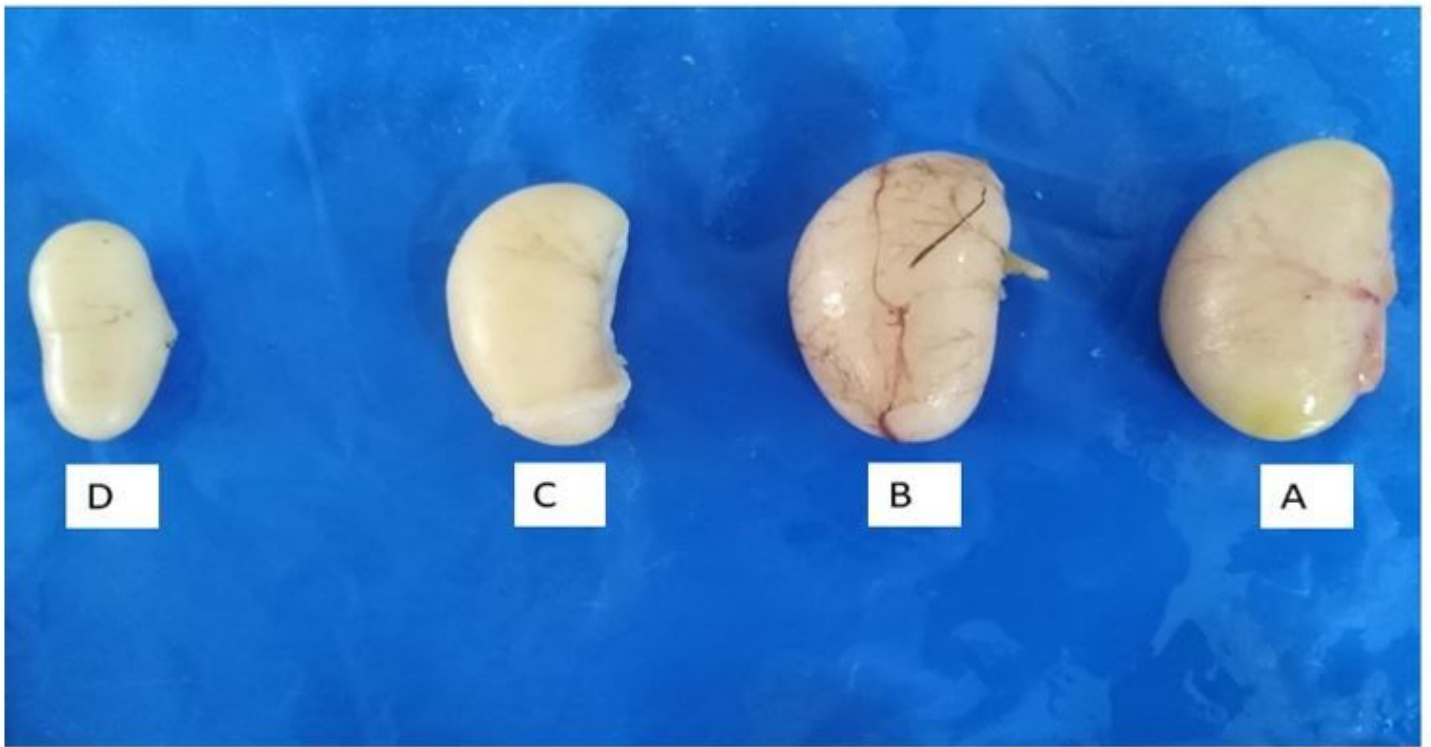


Figure 4

Photograph showing normal testes (A) and testicular atrophy in aflatoxin feeding groups (B-D) Control group= A, AFB1 100ppb = Group B, AFB1 200ppb = Group C, AFB1 400ppb = = Group D

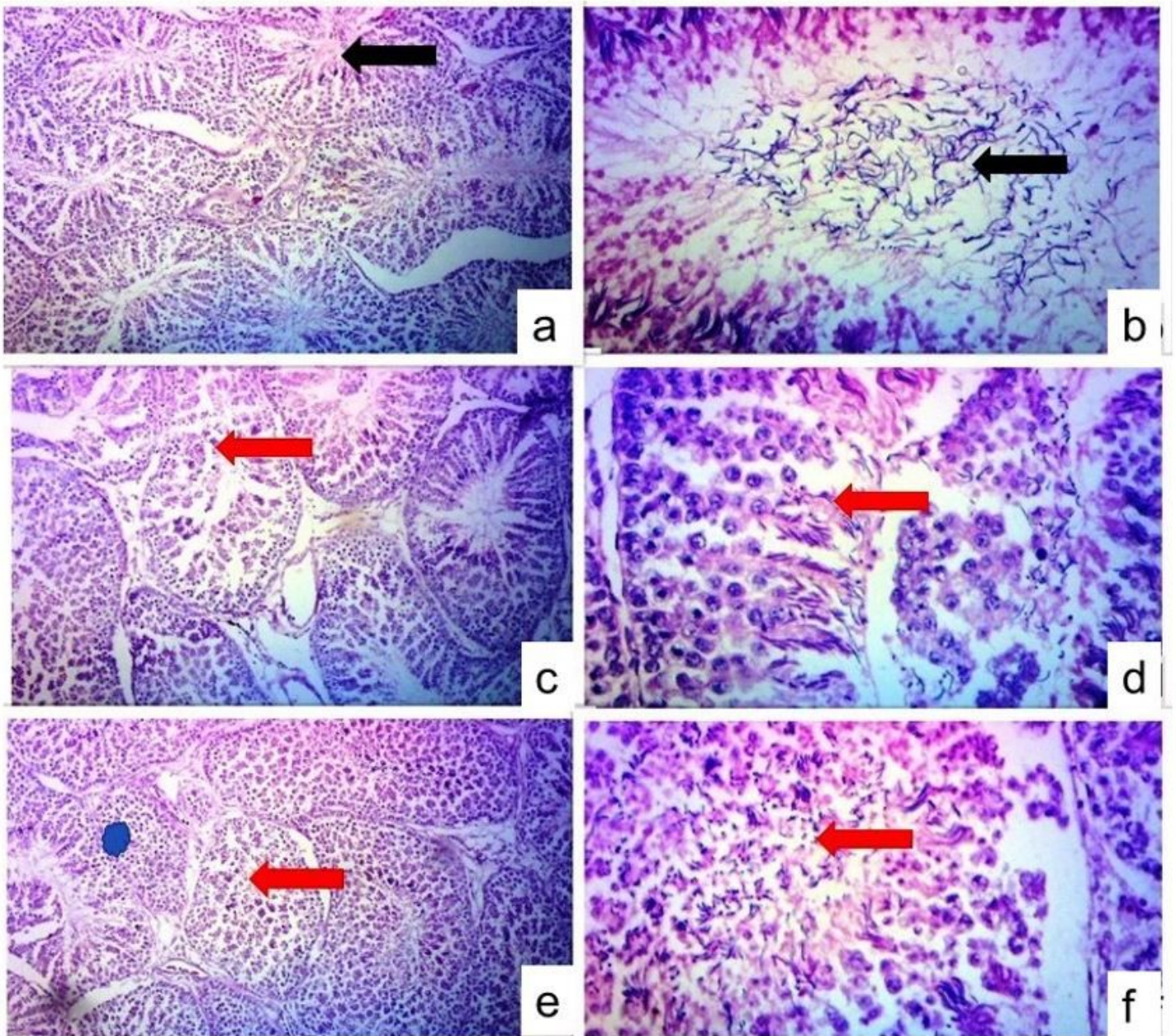


Figure 5

Photomicrograph of testes (5a & b) in control group showing patent seminiferous tubule with active spermatogenesis, bunches of mature spermatozoa indicated by black arrows, while 5c&d showing testicular parenchyma in group B having few spermatozoa in lumen of seminiferous tubule. Photomicrograph (5e&f) of testicular parenchyma in group D showing arrested spermatogenesis and nonpatent lumen of seminiferous tubule (blue steric) while red arrows indicating spermatids and pyknotic nuclei (H&E Staining: Fig.5a,5c,5e 100X & Fig. 5b, 5d, 5f 400X).