RESEARCH ARTICLE

Pathological effects of graded doses of aflatoxin B1 on the development of the testes in juvenile white leghorn males

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Abstract
Current experiment was planned to investigate the deleterious effects of the graded doses of aflatoxin B1 (AFB1) on white leghorn male birds. For this purpose, one-hundred birds of 8 weeks of 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; groups 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 the testes, and sperm motility were measured. Cellular immune response was observed through carbon clearance assay (CCA), P-HAP, and antibody response against sheep red blood cells (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 were observed at the end of the study. Grossly, testicular size and volume were reduced in AFB1 fed birds, while histological examination showed moderate to severe necrosis of testicular parenchyma, with partial to complete arrest of spermatogenesis. Very few sperms were found in group C, while they were almost absent in group D which was offered a diet containing 400 ppb AFB1. The motility of sperms was reduced in all treated groups except control. The abovementioned results showed that AFB1 had severe toxic effects on the reproductive and immunological parameters of WLH male birds in a dose-dependent manner.

Keywords Testes weight · Immune status · Histopathology of testes

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 (Saleemi et al., 2017; Imran et al., 2020; Gohar et al., 2020; Hernandez-Valdivia et al., 2021).

Aflatoxins are the metabolites produced from the Aspergillus sp. mainly A flavus and A parasiticus and further classified as aflatoxins B1, B2, G1, G2, M1, and M2 (Usman et al., 2019; Ali et al., 2021). 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 turkey 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 and 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). The 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 contaminated feed for a longer period resulted in testicular degeneration (Maryam & 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 changes drastically the hematological parameters (Hb, PCV, TEC, and TLC), and it also affects the serum biochemistry (Afzal and Saleem, 2004). Aflatoxin not only lowers 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; Ulucan et al., 2021).

In poultry, male infertility is the major problem especially at breeder flocks where artificial insemination is used 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 the following:

- Adverse effects of graded doses of aflatoxin B1 on male reproductive system functions and immune status in juvenile white leghorn males
- 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 having the age of 8 weeks 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 (25 birds/group and each group has five replicates with 5 birds in each replicate), namely, groups A, B, C, and D. The time frame of the trial was 10 weeks. Humidity and temperature in shed were maintained by installing the cooling pads and fans in experimental shed to maintain the relative humidity 70%, and temperature ranges from 24 to 26 °C. 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 was prepared. The feed for commercial white leghorn layers was formulated with standard formulation having crude protein (CP) 16% and metabolizable energy (ME) 2800 kcal/kg feed. The AFB1 level in basal diets was > 20 ppb as standard, and then the 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, were maintained and inoculated on sterilized potato dextrose agar and left for incubation for 7 days at 27 °C. These cultures were used for the production of aflatoxins by inoculating on rice by the method of Shotwell et al. (1966) and 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 (60th 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 (%) = \( \frac{(Abs. \text{ org weight (g)})}{(body \text{ weight (g)})} \times 100 \)

**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 5 µL was placed on a microscopic slide and observed under CASA. Blood was collected at the end of the experiment (60th day of experiment) for hormonal profile of serum testosterone, prolactin, and luteinizing hormone 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 interdigital space between the 3rd and 4th digits in 3 birds from each group on the 40th day of the experiment. The thickness of interdigital space was measured after 24 and 48 h after the initial administration of avian tuberculin (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 3000 g for 30 min, the Pelikan 4001 ink was centrifuged for the collection of supernatant fractions, and in 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 min and 3 min and 15 min of after injection, blood from wing vein (left side) was collected (200 µl/chick). Immediately after collection, 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 3 and 15 min as of that of 0 min.

\[ \text{Absorbance (µm)} = \frac{\text{Absorbance at time 0 min} - \text{Absorbance at specific time}}{\text{Absorbance at time 0 min}} \times 100 \]

**Gross and microscopic pathology of the testes**

The testicular tissues of the birds that died during the experiment or were euthanized and observed for gross lesions at day 30 and 60 of the experiment. The tissues including the 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 group means were compared by Duncan’s multiple range test (DMR test) by using MSTAT-C statistical software.

**Results**

**Clinical signs and behavioral alterations**

Clinical signs including alertness of the birds to signal, activity of the birds, and crowing behavior on getting mature were 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 shown 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 (Table 2). Groups A to C showed nonsignificant difference with each other. In the testes, the weight of all four groups followed the trend as A > B > C > D. Testes volume was significantly \( p \leq 0.05 \) higher in control group and the lowest in group D and also differed with groups B and C. In the testes, the volume of all four groups followed the trend as A > B > C > D.
### Table 1: Clinical signs and behavioral alterations of WLH breeder males fed with AFB1 intoxicated feed

| Weeks | Behavioral changes shown by birds | Score | Groups               | AFB1 100 ppb | AFB1 200 ppb | AFB1 400 ppb |
|-------|-----------------------------------|-------|----------------------|--------------|--------------|--------------|
|       |                                   |       | Control              | 0–4          | 0–4          | 0–4          |
| 1     | Alertness of the bird             | 4     | 4                    | 4            | 4            | 4            |
|       | Mating behavior                   | 0     | 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            |
| 2     | Alertness of the bird             | 0–4   | 4                    | 4            | 4            | 3            |
|       | Mating behavior                   | 0–4   | 0                    | 0            | 0            | 0            |
|       | Crowing                           | 0–4   | 2                    | 1            | 1            | 2            |
|       | Foaminess in the droppings        | 0–4   | 1                    | 1            | 1            | 1            |
|       | Total                              |       |                      | 7            | 8            | 6            |
| 3     | Alertness of the bird             | 0–4   | 4                    | 3            | 3            | 3            |
|       | Mating behavior                   | 0–4   | 1                    | 1            | 0            | 0            |
|       | Crowing                           | 0–4   | 3                    | 2            | 2            | 1            |
|       | Foaminess in the droppings        | 0–4   | 1                    | 1            | 1            | 1            |
|       | Total                              |       |                      | 9            | 7            | 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                              |       |                      | 9            | 8            | 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            |
| 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            |
| 7     | Alertness of the bird             | 0–4   | 4                    | 3            | 3            | 2            |
|       | 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           |
|       | Grand total                        |       |                      | 112          | 71           | 57           |

### Table 2: Absolute organ weight of WLH male layers fed with different levels of aflatoxin B1 after 1st slaughtering and 2nd slaughtering

| Groups          | 1st slaughtering |         | 2nd slaughtering |         |
|-----------------|------------------|---------|------------------|---------|
|                 | Testes weight    | Testes volume | Testes weight    | Testes volume |
| A (control)     | 8.525 ± 0.39a    | 9.250 ± 1.06a | 7.350 ± 0.21a    | 7.175 ± 0.46a |
| B (AFB1-100 ppb)| 7.500 ± 0.42a    | 7.625 ± 0.18b | 6.500 ± 0.00ab   | 6.500 ± 0.71ab |
| C (AFB1-200 ppb)| 7.332 ± 1.18a    | 6.750 ± 0.35c | 5.105 ± 0.89ab   | 4.750 ± 0.85b |
| D (AFB1-400 ppb)| 4.700 ± 0.36b    | 5.275 ± 0.32d | 4.393 ± 1.57b    | 4.075 ± 1.45ab |

Values (mean ± SD) in each column followed by different letters are significantly different ($p \leq 0.05$)
In the 2nd killing, testes weight was significantly higher in control group A. In group D, testes weight was significantly lower as compared to control group. Testes volume of group A was significantly higher as compared to group D while nonsignificant with groups B and C.

**Relative organ weight of WLH male layers**

In the 1st killing, the relative organs weight of the testes of group D was significantly lower as compared to control group A. The trend seen in testes weight was as follows: 

A > B > C > D. In the second killing, there was similar trend as observed in the first killing (Table 3).

**Antibody titers against sheep red blood cells (SRBCs)**

Antibody titer of total antibodies at the 7th day of the initial dose of group A and group B was nonsignificant with each other and significantly higher as compared to groups C and D, while group D showed the lowest antibody titer on the 7th day. The IgM titers were nonsignificant among all groups, but group D showed the least value of IgM, and group A showed the maximum value of IgM on the 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 (Fig. 1a).

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 nonsignificant 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 nonsignificant 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 Fig. 1b.

| Table 3 | Relative organ weights of WLH males fed with different levels of aflatoxin B1 on 1st slaughtering and 2nd slaughtering |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| Groups | 1st slaughtering | 2nd slaughtering |
|        | Testes weight | Testes volume | Testes weight | Testes volume |
| A (control) | 0.509 ± 0.01a | 0.551 ± 0.03a | 0.475 ± 0.11a | 0.465 ± 0.12a |
| B (AFB1-100 ppb) | 0.470 ± 0.04a | 0.477 ± 0.01ab | 0.372 ± 0.00a | 0.372 ± 0.04a |
| C (AFB1-200 ppb) | 0.455 ± 0.09a | 0.418 ± 0.03bc | 0.317 ± 0.08a | 0.295 ± 0.07a |
| D (AFB1-400 ppb) | 0.308 ± 0.02b | 0.346 ± 0.03c | 0.295 ± 0.05a | 0.275 ± 0.05a |

Values (mean ± SD) in each column followed by different letters are significantly different (p ≤ 0.05)
Mononuclear phagocytic response carbon clearance assay

In vivo phagocytic response of mononuclear cells to carbon particles at 3-min response groups had nonsignificant difference among all groups. At 15 min, 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 min 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 h, 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-h response. At response time 48 h, control group A was significantly higher as compared to all groups. Group B and group C were nonsignificant to each other, while both groups showed response significantly higher as compared to group D. At response 72 h, 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 the dose of toxin.

Gross lesions

There was no obvious gross lesion seen in the control group. The 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 AFB-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 and 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 and 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 and 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. The number of spermatozoa was less; necrotic changes were also present in seminiferous tubule (Fig. 5e and f).

**Discussion**

The poultry industry is a vital source of balanced diet in poor masses in developing countries 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 Ortatatli and Oguz, (2001), Liu et al. (2018), and Wang et al. (2018). 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) which 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 weeks of experiment, there was increase in the size of the 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), Daniel et al. (2009), and Cho et al. (2021).

Sperm motility also showed a regular trend as it decreased from groups 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 in chickens (Kurniasih and Prokoso, 2019; Sineque et al., 2017).

### Table 4 Motile sperms percentage of each ejaculate

| 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 eachrow followed by different letters are significantly different ($p \leq 0.05$)

### Table 5 Levels of reproductive hormones after final killing

| Groups     | Testosterone (ng/mL) | Prolactin (mIU/mL) | LH (mIU/mL) |
|------------|----------------------|--------------------|-------------|
| A (control)| 2.484±0.39a          | 0.065±0.1d         | 0.014±0.00a |
| B (AFB1-100 ppb) | 2.347±0.52a          | 0.230±0.6c         | 0.045±0.06a |
| C (AFB1-200 ppb) | 0.933±0.13b          | 0.522±0.06b        | 0.017±0.01a |
| D (AFB1-400 ppb) | 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 ($p \leq 0.05$)

[**Fig. 4** Photograph showing normal testes (A) and testicular atrophy in aflatoxin feeding groups (B–D). Control group, A; AFB1 100 ppb, group B; AFB1 200 ppb, group C, AFB1 400 ppb, group D]
Different reproductive hormones like testosterone, LH, and prolactin also followed the same trend. Control group has the highest level of reproductive hormones such as testosterone, LH, and prolactin; however, least levels of these hormones were observed in group D. Similar findings were also reported by Elsayed et al. (2019), Mai et al. (2020), Abdel-Haq et al. (2000), Adedara et al. (2014), and Hasanzadeh et al. (2011); Ijaz et al. (2020) also reported this similar kind of results.

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, rats, animals, and rabbits (Bbosa et al., 2013; Hasanzadeh et al., 2011; Zhang et al., 2021; Zubair et al., 2021). This reduction in the level of testosterone may be attributed to reduction in the number of Leydig cells. There was no significant decrease on the 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 in tubule having patent lumen and normal appearance of seminiferous tubule. All the stages of spermatogenesis are also present. In 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. The number of spermatozoa was less indicating partial arrest of spermatogenesis. At few places, the 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 ours were observed by Ahmed et al. (2012), Adedara et al. (2014), Ewuola et al. (2014), Supriya et al. (2014), Kourousekos and Theodosiadou (2015), Murad et al. (2015), and Pasha et al. (2021). The results of the present study concluded that AFB1 intoxication leads to a decrease in body weights, feed intake in dose-related manner. The gross and microscopic changes in the aflatoxin groups were more pronounced. There was a marked decrease in testosterone levels and other hormones in a dose-dependent manner from group A to D. Similar results were observed by Mukuma et al. (2016),
The results of the present study concluded that AFB1 intoxication leads to a 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. It may be concluded from this study that exposure of mycotoxins in continuous and increasing manner leads to a decrease in the infertility of juvenile white leghorn males. This reduction in the fertility of the male requires the protective measures to save the poultry industry from aflatoxins contamination.

Author contribution Experimental design and analysis: AA, MKS, MM, and STG.

Histopathology: MKS and STG.
Investigation: FM, SAB, MRH, MI, HI, IZ, IA, and AR.

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Data availability It is also declared that data and material be online.

Declarations

Ethics approval 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 agreed to participate in editing this article.

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

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