

Full Length Research Paper

Effects of aflatoxin B₁ (AFB₁) on follicle stimulating hormone, luteinizing hormone, testosterone, and estradiol hormone levels in reproductively mature male pigs

Mukumu C. K.^{1*}, Macharia B. N.², Lusweti D.¹ and Mwaniki D. M.³

¹Department of Biological sciences, Moi University, School of Biological and Physical Sciences, P. O. Box 3900, Eldoret 30100, Kenya.

²Department of Human Pathology and Forensic Medicine, Moi University, School of Medicine, P. O. Box 4606, Eldoret 30100, Kenya.

³Department of Biological Sciences, University of Eldoret, School of Science, P. O. Box 1125-30100, Eldoret, Kenya.

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Aflatoxin is a major food contaminant, with adverse effects on the physiology of both humans and animals. Exposure to aflatoxins has been known to pose a great threat to both humans and animals, particularly, in the tropics, with varied pathophysiological effects. This research focused on the effects of aflatoxin B₁ on the pituitary-gonadal axis of pigs, an area that has not fully been investigated since most studies have dwelt on other experimental animals. The aim of this study was to determine the effects of aflatoxin B₁ on follicle stimulating hormone, luteinizing hormone, testosterone, and estradiol hormone levels in reproductively mature male pigs. This research adopted an experimental design using twelve reproductively mature Large White pigs aged 7 to 9 months and of average body weight of 54 kg. The pigs were allowed to acclimatize for a period of seven days. AFB₁ was obtained from Bora Biotechnology Company in Nairobi and the doses were given in three levels in the ratio of 1:2:3. The 1st treated group received 80 ppb per day, 2nd treated group received 160 ppb and the 3rd treated group received 240 ppb per day for 60 days. The control group received aflatoxin-free diet for the same study period. This was orally given as predetermined aflatoxin levels mixed with 150 g of pig finishers feed served to each pig in a separate aluminum feeding pot each in their specific experimental group. Each pig was thereafter served its daily 2 kg Unga feed in separate feeding pot. Water was also provided *ad libitum*. Five milliliters of blood samples were collected once every week from either the right or left ear of the pig in vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) which is an anticoagulant. Plasma samples were prepared by incubating the whole blood at room temperature for 10 to 20 min. The tubes were centrifugated for 20 min at 2,000 to 3,000 rpm. The supernatant was carefully collected and stored in a freezer at -21°C for hormonal assays. Consumption of increasingly higher doses of aflatoxin B₁ by pigs led to decreased levels of luteinizing hormone, testosterone and estradiol in a dose-dependent manner. The level of follicle stimulating hormone was however not significantly affected by consumption of aflatoxin B₁ dosages given.

Key words: Aflatoxin B₁, follicle stimulating hormone, luteinizing hormone, testosterone, estradiol hormone, pig.

INTRODUCTION

Aflatoxin (AF) is one of the contaminants in foods and feeds, with varying effects on the physiology of both animals and humans. It is a mycotoxin, a secondary metabolic by-product of the toxigenic fungi mainly *Aspergillus flavus* and *Aspergillus parasiticus* during their natural metabolic processes (Ahmed et al., 2012). The four major groups of aflatoxin that have been described based on their fluorescence at chromatography are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂).

The two main *Aspergillus* species yield different secondary metabolites such that AFB₁ and B₂ are produced by *A. flavus* and *A. parasiticus* while AFG₁ and G₂ are produced by *A. parasiticus*. In this classification, “B” and “G” stand for blue and green fluorescent colors, respectively (Ajani et al., 2014). The subscript numbers 1 and 2 indicate major and minor compounds, based on the degree of toxicity. AFB₁ is the most common, most toxic and a known carcinogen (Quintana et al., 2012).

Aflatoxins are not transmissible between animals, thus, the main cause of the toxicity in humans and animals is consumption of contaminated food and feed stuff. The toxigenic fungi can contaminate various feed components like maize, rice, wheat, peanuts, millet, oily feedstuffs, some of which form the majority of common food for both humans and animals. This contamination is dependent on the prevailing environmental conditions such as temperature range of between 28 and 33°C, water activity of about 0.83 to 0.97_{a_w} and oxygen availability which favour production of aflatoxin in feeds (Bagheri et al., 2014). Contamination of animal feeds with aflatoxin can occur during the growth of the crop while in the field, at harvest and during postharvest operations as well as in storage. The task of preventing the occurrence of this toxins in swine feeds is therefore a very challenging one (Kanora and Maes, 2009).

Aflatoxins, particularly AFB₁, have been shown to impact on the endocrine glands and reproductive system at varying degrees, both in experimental animals and in humans. Hasanzadeh et al. (2011) did a study to determine the effects of aflatoxin B₁ on profiles of gonadotropic (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]), steroid (testosterone and 17β-estradiol) and prolactin hormones in adult male rat. The results showed that the level of FSH significantly increased in all treatment groups fed on aflatoxin contaminated food. The levels of luteinizing hormone and testosterone were lower in all of the treated groups, while prolactin was significantly higher in the treated groups.

The level of 17β-estradiol was significantly decreased only in the group that received higher concentration of aflatoxins in the diet. Their findings explain the hypophysotoxicity of aflatoxin and in particular on adenohipophysitis. Effect of the toxin on the hypophysis could have led to the decreased level of the luteinizing hormone. The increased level of FSH in serum was attributed to the degeneration and desquamation of the epithelium, and a decrease in the size and thickness of the germinal layer in the seminiferous tubules. Damage to testes and a resulting reduction in circulating inhibin-B tend to elevate serum FSH levels. Serum testosterone levels were reduced mainly due to extreme damage to Leydig's cells. The level of 17 β-estradiol in the serum decreased only by administration of a relatively high dose of aflatoxin.

Clarke et al. (1987) investigated on endocrine and testicular related changes in male chicken fed on varying concentrations of dietary aflatoxin. Male chicken showed reduced plasma testosterone levels following the ingestion of aflatoxin contaminated diet, compared to the control group. Together with the reduced plasma testosterone concentrations were changes in testicular weight. Levels of plasma luteinizing hormone were significantly reduced in aflatoxin-fed males compared to the control group.

Ewuola et al. (2014) also reported lower levels of testosterone in aflatoxin-fed goats an effect that could be attributed to impairment of Leydig cells by the increased toxic levels. This may also explain the reduced spermatozoa production in the testis.

METHODOLOGY

This research adopted an experimental design using twelve reproductively mature Large White pigs aged 7 to 9 months and of average body weight of 54 kg. They were obtained from the University of Nairobi and housed in a pig pen at Karen in Nairobi. Completely randomized design was used in the allocation of the animals to the control group and to three treatment groups, each group comprising of three pigs. The pigs were allowed to acclimatize for a period of seven days. AFB₁ was obtained from Bora Biotechnology Company in Nairobi and the doses were given in three levels in the ratio of 1:2:3. The 1st treated group received 80 ppb per day, 2nd treated group received 160 ppb and the 3rd treated group received 240 ppb per day for 60 days. The control group received aflatoxin-free diet for the same study period. This was orally given as predetermined aflatoxin levels mixed with 150 g of pig finishers feed served to each pig in a separate aluminum feeding pot each in their specific experimental group. Each pig was thereafter served its daily 2 kg Unga feed in separate feeding pot.

*Corresponding author. E-mail: agnesmukumu@gmail.com.

Table 1. Partial regression of FSH on dose, age, and weight.

Variable (FSH)	Slope and Intercept	Standard error	T-Statistic	P-value
Intercept	15.9924	2.90713	5.50111	0.0000
Weight	-0.20247	0.048384	-4.18463	0.0001
Age	0.009233	0.006579	1.40355	0.1645
Dose	-0.00137	0.00229	-0.5971	0.5522

$$\text{FSH} = 15.9924 - 0.20247 \times \text{Wt} + 0.00923347 \times \text{Age} - 0.00137 \times \text{Dose}.$$

Table 2. Partial regression of LH on age, weight and toxin dosage.

Variable (LH)	Slope and Intercept	Standard error	T-Statistic	P-Value
Intercept	2.22118	3.80107	0.584357	0.5607
Weight	0.005879	0.063262	0.092927	0.9262
Age	0.009138	0.008602	1.06236	0.2914
Dose	-0.00617	0.002995	-2.06105	0.0427

$$\text{LH} = 2.22118 + 0.005879 \times \text{Wt} + 0.009138 \times \text{Age} - 0.00617 \times \text{Dose}.$$

Table 3. Partial regression of estradiol on age, weight and toxin dosage.

Variable (E2)	Slope and Intercept	Standard error	T-Statistic	P-Value
Intercept	102.143	38.2333	2.67157	0.0092
Weight	-0.72039	0.635806	-1.13303	0.2607
Age	0.014699	0.085704	0.171507	0.8643
Dose	-0.21256	0.030091	-7.0638	0.0000

$$\text{Estradiol} = 102.143 - 0.72039 \times \text{Wt} + 0.014699 \times \text{Age} - 0.21256 \times \text{Dose}.$$

Water was also provided *ad libitum*. Five milliliters of blood samples were collected once every week from either the right or left ear of the pig in vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) which is an anticoagulant. Plasma samples were prepared by incubating the whole blood at room temperature for 10 to 20 min. The tubes were centrifugated for 20 min at 2,000 to 3,000 rpm. The supernatant was carefully collected and stored in a freezer at -21°C for hormonal assays. Analysis of hormones was carried out at the University of Nairobi, Department of Clinical Studies. The reproductive hormones analyzed were the porcine FSH, porcine LH, porcine testosterone and porcine Estradiol (E2). Porcine ELISA kits for each specific hormone were obtained from *In-vitro* Diagnostics (EA) Limited, Nairobi. These kits were used in quantitative determination of the concentration of each specific hormone. Since the animals used were of different weight and age, these parameters were factored in as variables and statistical controls were used which gave the same results as experimental controls. The data was subjected to multiple regression analysis.

RESULTS

Porcine FSH

Table 1 shows the slopes of the partial relationship of FSH on dose, weight and age. Table 1 also shows that

weight had a relationship with FSH. FSH decreased with increase in weight ($P < 0.05$). However, FSH had no significant relationship with age and the toxin dosage.

Porcine LH

Table 2 shows the slopes of the partial relationship of luteinizing hormone on dose, weight and age. Weight and age had no relationship with luteinizing hormone concentration ($P > 0.05$). Only aflatoxin dosage affected luteinizing hormone significantly ($P < 0.05$) in which case luteinizing hormone decreased significantly with increased aflatoxin level.

Porcine estradiol (E2) hormone

Table 3 shows the slopes of the partial relationship of estradiol on dose, weight and age. Table 3 also shows that the aflatoxin dosage given had a highly significant effect on the level of estradiol hormone ($P < 0.05$), while the age and weight of the pigs had no relationship with

Table 4. Partial regression of testosterone on age, weight and toxin dosage.

Variable (Testosterone)	Slope and Intercept	Standard error	T-Statistic	P-Value
Intercept	444.984	141.809	3.13791	0.0024
Weight	-5.53623	2.36016	-2.3457	0.0216
Age	0.13458	0.320905	0.419375	0.6761
Dose	-0.54583	0.11173	-4.88526	0.0000

$$\text{Testosterone} = 444.984 - 5.53623 * \text{Wt} + 0.13458 * \text{Age} - 0.54583 * \text{Dose}.$$

estradiol hormone ($P > 0.05$). Estradiol concentration decreased significantly with increased aflatoxin level.

Porcine testosterone hormone

Table 4 shows the slopes of the partial relationship of testosterone on dose, weight and age. Both weight and aflatoxin B₁ dosage had a significant impact on testosterone level ($P < 0.05$). The relationship between the toxin dosage and testosterone was highly significant as shown in the table. Testosterone concentration decreased with increase in animal weight and with increase in aflatoxin dosage. However, age had no significant relationship with testosterone hormone ($P > 0.05$).

DISCUSSION

The plasma levels of luteinizing hormone, testosterone and estradiol of the aflatoxin challenged groups were significantly reduced in a dose related manner compared to the control group. These findings agree with those of studies carried out on other experimental animals. The results in our study agree with those of Ewuola et al. (2014) who found out that goats fed higher aflatoxin levels had significantly lower testosterone levels compared to the control group. Clarke et al. (1987) had earlier found similar results in male chicken fed on varying levels of dietary aflatoxin. Their results showed reduced plasma testosterone levels, as well as reduced levels of plasma luteinizing hormone in the aflatoxin treated groups compared to the control group. Plasma testosterone levels have been shown to reduce in white leghorn male chicken and in birds fed varying levels of aflatoxin contaminated feed as described by Bbosa et al. (2013). The results of this study are also comparable to the findings of Hasanzadeh et al. (2011) who reported that aflatoxin B₁ causes reduction in the concentration of testosterone as well as 17 β -estradiol in aflatoxin treated male rats. Our study findings of reduced levels of testosterone and estradiol may be attributed to reduction of Leydig cells as well as degeneration of Sertoli seen in

examined testicular tissues of the studied pigs. Bbosa et al. (2013) suggested that one of the most common mechanisms for the action of AFB₁ is the binding of DNA to form complexes and inhibit nucleic acid synthesis. This mode of action may explain the direct effect of aflatoxin B₁ on Leydig cells and Sertoli cells in the testes, and consequently, the reduction of the gonadal hormones, namely, testosterone and estradiol. The aflatoxin dosages given to the pigs did not have any significant effect on the level of FSH. However, luteinizing hormone was significantly reduced by aflatoxin in a dose-related manner. These findings differ partly from the findings of Hasanzadeh et al. (2011) who reported a significant increase in the levels of FSH, but are in agreement regarding the reduced level of LH in aflatoxin fed male rats. The reduced luteinizing hormone levels can be explained by hypophysotoxic effects of aflatoxin especially on adenohypophysis (Hasanzadeh et al., 2011). Regulation of FSH is similar to that of LH except for the specific inhibitory effect of inhibin B on the production and secretion of FSH. In our larger study, testicular tissues of the AFB₁-treated pigs showed reduced number of Sertoli cells, an effect that would consequently translate to reduced levels of inhibin B. Decline in inhibin B level reduced its inhibitory effect on FSH. This mechanism is expected to cause an elevated FSH level. The FSH level in AFB₁-treated pigs did not differ significantly from the control group. This is explained by the hypophysotoxic effects of AFB₁ which is known to lower the FSH level (Mabeck et al., 2005). Weight as an extrenous variable was shown to have a significant effect on the levels of FSH and testosterone hormones a finding that may call for further investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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