

EFFECT OF AFLATOXIN M1, “A HYDROXYLATED METABOLITE OF B1” ON SPERM CELL QUALITY OF ADULT MALE WISTAR RATS

¹Ibeh Nnanna Isaiah, ²Taidi Ekkrakene, ³Okungbowa MO, ⁴Otabor Florence, ⁴Omorodion Nosa Terry

^{1,4}Health Services Department, Medical Laboratory Science Unit, University of Benin, Benin City Edo state Nigeria, PMB 1154.

²Faculty of Basic Sciences, Department of Microbiology, Benson Idahosa University, Edo state Nigeria.

³Faculty Basic Medical Sciences, Department of Medical Laboratory Sciences, University of Benin, Benin City, Nigeria.

Abstract

The effect of aflatoxin on reproductive health takes a systematic approach as it indicates that a significant number of people living in the third world regions are exposed to these toxins due to the poor storage of grains. To expose the effects of aflatoxin M1 a hydroxylated metabolite of aflatoxin B1. Twenty four Adult male Wistar rats were acclimatized and broken down into four groups, Test Group one (Two weeks exposure), Group Two (four weeks exposure), Group Three (Six weeks exposure) and Group Four (Control Group). The Test Group 1, 2 and 3 were orally administered 7.2g/kg (LD50) of Aflatoxin M1 for the desired weeks of exposure and the sperm cell quality was assessed to determine the effect of Aflatoxin M1 on sperm cell quality in Adult male Wistar rat. The predetermined level of sperm cell quality is its ability to be motile as the motility of the sperm cell determines its viability. Group three had the highest presence of sluggishly immotile cells with very low progressive motility, followed by group, two and one, 37.5± 9.97, 55±9.91, and 71.67±3.07 as against the control groups 85.8±2.17 with the highest significance at the group three which showed an extended exposure of aflatoxin m1 (p=0.05). This study links infertility and aflatoxin consumption, as the higher the doses the more spermatotoxicity is built which invariably could lead to sterility and azoospermia.

Keywords: Aflatoxin M1; Sperm cell quality; Rats.

Introduction

Aspergillus is a known fungus which is mostly found as a flora in grain storage, it has its optimal growth at 25°C with very minimal water activity which is about 0.75. Its secondary metabolites are produced at 10-12°C, the most toxic metabolites have their optimal temperatures at 25°C with water activity of about 0.95 [1]. These secondary metabolites are known as ‘Aflatoxin’ (AF) belongs to a group of mycotoxins which are produced in large numbers by the wide family of *Aspergillus* species, although there are basically three phylogenetic distinct sections. The main producers of this toxins are, *Aspergillus flavus* and its counterpart *Aspergillus parasiticus* but much more distinctive there have been toxin production by *Aspergillus nomius*, *Aspergillus parvisclerotigenus*, *Aspergillus bombycis* and *Aspergillus pseudotamarii*, of section Flavi, *Aspergillus rambellii* and *Aspergillus ochraceoroseus* from *Ochraceorosei* and *Emericella astellata* and *E. venezuelensis* [2]. They are known to contaminate a broad section of the global food which includes, maize, rice, sorghum, barley, rye, wheat, groundnut, peanut, soya, cottonseed and most other product which are processed from these primary feedstuffs in low income countries [3-6]. Aflatoxin have been known to cause health problems throughout history. 1960 unveiled the significance of its contamination within the agricultural sector in that year the toxin was isolated and identified as the cause of

“Turkey X-disease” after the death of over 100,000 turkeys which died from liver necrosis and hyperplasia in England, this occurred after the poultry birds had consumed groundnuts infected with *Aspergillus flavus* [7-10] also documented and estimated by Williams et al. in 2004 that about 4.5 billion of the world’s population are exposed to the toxic aflatoxins owing to the fact that they are commonly found everywhere. These essential factors that contribute to aflatoxin wide contamination of food stuff include the climate of the region, the crop planted and its genotype, the soil type and the maximum, minimum temperatures coupled with the net daily evaporation [11]. Aflatoxin M1 (AFM1) which is a hydroxylated metabolic derivative of AFB1, AFM2 is a metabolic derivative of AFB2; both are gotten from the metabolism of some animals, and are usually found in breast milk and urine [12- 18]

It has been reported that aflatoxins, once ingested (because of their low molecular weight), are rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism, and then quickly appear as metabolites in blood after just 15 minutes and in milk as soon as 12 hours post-feeding [19-24]. Recent studies also suggest that the B aflatoxins may cause neural tube defects in populations that consume maize as a staple food (Wild & Gong, 2010). Aflatoxin B1 and its derivative are also known to cause secondary infertility both in males and female rabbits and rats [25- 30].

Furthermore, due to this important worldwide issue, some organizations and institutions have been purposing a great number of practical primary and secondary prevention strategies, especially for developing coun-

eISSN: 2523-6709
pISSN: 2523-6695
DOI: 10.31878/ijcrpp.2019.32.2

Correspondence: Ibeh Nnanna Isaiah, Department, Medical Laboratory Science Unit, University of Benin, Benin City Edo state Nigeria, PMB 1154 Email: nnanna.ibeh@uniben.edu

tries, in order to reduce the risks given by this public problem, but they could be beneficial if political wills and financial investments are applied to what remains a largely ignored worldwide health matter.

The aim of this study is to determine the effect of Aflatoxin M1 effects on Sperm cell quality in adult male Wistar rats, its toxico-kinetics and sperm toxicity.

Material and Methods

Study Site: The University of Benin Animal house in The Department of Anatomy was used as the study site where the male adult Wistar rats were acclimatized and orally administered the fungal toxin.

Study Animals: Twenty four Male Wistar (albino) rats 350-470g of reproductive age were obtained. These test animals were kept at room temperature (25±2°C) under a 12L:12D cycle and were provided with water and rat standard diet (Altromin GmbH, Large) ad libitum. The experimental studies were conducted in accordance with the ethics of the usage of laboratory animals [31-36].

Experimental Design: A randomized block design was used in this analysis. Rats were broken down into groups and subgroups with the treatment plan across the groups. Each group signified two weeks of exposure per day of aflatoxin M1 of 7.2mg/kg per body weight. Twenty four Adult male Wistar rats were acclimatized and broken down into four groups, Test Group one (Two weeks exposure), Group Two (four weeks exposure), Group Three (Six weeks exposure) and Group Four (Control Group). The Test Group 1, 2 and 3 were orally administered 7.2g/kg (LD50) of Aflatoxin M1 for the desired weeks of exposure and the sperm cell quality was assessed to determine the effect of Aflatoxin M1 on sperm cell quality in Adult male Wistar rats.

Treatment of Male Rats: Aflatoxin M1 (Sigma) was given orally with a dosage of 7.2mg/kg per day for 42 days (6 weeks) in a volume of 0.14ml vehicle (demineralized water containing 0.2% per 100g body mass). Fertility of the male Wistar rats was assessed after dietary Aflatoxin M1 by looking at the sperm cell quality [37-39].

Sperm Cell Morphology and Count

Isolation of Sperm Cells: Sperm cells were collected from the vas deferens of the sacrificed rats; the rats were sacrificed and the vas deferens located and ligated with a minimum of 36mm length, both extremities of the vas deferens was ligated, cut and placed in a sterile petri dish. To the petri dish, 6 µl of normal saline already adjusted to 37±2°C was added. The Vas deferens was teased to allow the sperm cell to diffuse out of it. A drop of the sperm cell from the petri dish was placed on a grease free clean slide and covered with a transparent cover slip [37, 39].

Motility of Spermatozoa: The sperm cell motility was determined with the correlation between progressively motile sperm cells after ejaculation and the fertility. The motility was evaluated with regards to three variables: Progressively motile, Non-progressive motility and immotile spermatozoa and it is usually expressed in percentile. Spermatozoa can show good motility and viability in the seminal plasma 24 hours after ejaculation but in some semen samples the motility may decline much

faster [37-40].

A drop of the sperm cell was taken from the petri dish and dispensed on a clean grease free slide and further covered with a transparent cover slip. The slide was placed on the microscope and viewed with the x20 and x40 objective magnification lens. The motility was scored in percentage according to their nature of motility as, Progressive, Non-progressive and immotile sperm cells [18, 24, 41].

Vitality Testing: One volume of semen (a drop) was mixed into two volumes of eosin solution (1% diluted water). After 30 seconds three volumes of nigrosine solution (10% nigrosine) was added and the sample homogenized. A thin smear was then made immediately and air dried. The Stained slide was examined under the oil immersion objective lens (x100). Live spermatozoa were unstained (white) and the dead ones were red [42-44].

Morphology: The sperm cell morphology was assessed by staining the slide with the Improved Eosin and Leishman stain [39, 40].

A drop of the sperm cells was dispensed on a grease free clean slides and a smear was made, the slide was left to air dry. The slide was flooded with the Improved Eosin and Leishman stain for 15 mins. The stain was rinsed and the back was blotted dry with cotton wool and left to air dry. The slide was placed in a microscope with the magnification lens at x100. The slide was viewed with at least 30 magnification field, the normal and abnormal sperm cells were spotted and scored in percentage.

Statistical Analysis: The SPSS software for the analysis of variance was used to determine the significant difference at ($p \leq 0.05$) for the effect of Dietary Aflatoxin M1 on sperm cell quality in adult male Wistar rats.

Results

The results of the effect of Aflatoxin M1 on Adult male Wistar rats revealed the negative effect on sperm cell quality as compared with the control group. The descriptive statistics of the sperm cell motility and count between and across the study groups; revealed that the highest and lowest progressively motile cells and the highest and lowest total sperm cell counts were 71.67±3.07, 37.50±9.97 and 280±24.36, 140±25.69 as compared with the control group of 85.83±2.73 and 378±15.15 respectively.

The results of sperm cell morphology and count between and across the study groups with the highest and lowest normal morphology with mean of 75.00±2.236 and 64.17±3.745 as compared with the control group 90.83±0.833.

For the progressively motile sperm cells, the number of motile cells dropped from 71.6% in rats after 2 weeks of being fed Aflatoxin diet to 55% in 4 weeks and then 37.5% in 6 weeks, compared to values of the control group not fed Aflatoxin diet with 85.8% progressively motile sperm cells.

The Non progressively motile sperm cells did not drop in rats after 2 weeks and 4 weeks of being fed Aflatoxin M1 diet with 11.6% respectively, but dropped to 10.8 % in rats after 6 weeks on dietary Aflatoxin M, compared to the control group with 5.8%.The Immotile sperm cells increased from 16.6% in rats after 2 weeks of Aflatoxin M1 diet to 35.0% in 4 weeks and then 53.3% in 6 weeks, compared to the control group with 8.3% .The Total sperm cell count dropped from 280x 10⁶cells/mm³ in rats after 2 weeks of Aflatoxin M1 diet to 206 x 10⁶cells/mm³ in 4 weeks and then 140 x 10⁶cells/mm³ in 6 weeks of being on Aflatoxin M1 diet, compared to the control group with 378 x 10⁶cells/mm³ respectively.

There was an increased levels of abnormal sperm cell from 25% in rats after 2 weeks of being fed Aflatoxin M1 diet to 30% in 4 weeks and 35.8% in 6 weeks, compared to values of the control group with 9.1% abnormality.

The presence of abnormal sperm cell formation as seen in rats 2 weeks of being fed Aflatoxin diet presenting with headless sperm cells, multiple tailed sperm cells, short tailed sperm cells respectively (Plate 1). In week 4 there was more abnormal sperm cells presenting with headless sperm cells, bent headed sperm cells, and tail-less sperm cells respectively (Plate 2). In week 6 six of exposure to dietary Aflatoxin M1, the marked abnormality presented were more of degenerated forms, co joined headed sperm cells and premature sperm cells (Plate 3), compared to the control group with no abnormal sperm cells as shown in (Plate 4).

Discussion

The spermatozoa harvested from rats dietary fed aflatoxin M1 showed rapid decrease in their motility with the increase in time of treatment with 71.67±3.07 in 2 weeks to 55.00±9.91 in 4 weeks and 37.5±9.97 in 6 weeks, compared to the control of 85.83±2.17 respectively. The differences in the average spermatozoa motility of the test Wister rats and the control were statistically significant (P<0.05). The depression of spermatozoa motility in rats exposed to aflatoxin M1 suggest that aflatoxin M1 impaired the metabolic activities of the sperm cell which is also reported in previous studies

[29, 30]. The adhesion of aflatoxin M1 to the nucleic acid of a sperm cell may inhibit the cells response to chemotactic agents thereby decreasing its motility (Patten, 1981). The loss of nucleic acids and certain enzymes by sperm cells may contribute to their low motility and viability [31, 32].

The immotile sperm cells also signify dead cells and dysgenesis was highest in the 6 weeks of exposure with 53.33±12.2 and the lowest immotile sperm cells was seen with 16.67±2.10 in 2 weeks of rats dietary exposed to Aflatoxin M1 as compared with the control with 8.33±1.054. Due to the active spermatotoxic effect of high accumulation of aflatoxin M1 in semen, this sets up an oxidative stress in the testicular region thus leading to the depletion of preformed antioxidants [33, 34].

In this study the Spermatozoa harvested from the rats decreased in sperm cell count over the period of exposure to dietary aflatoxin M1. This difference is expressed in the average spermatozoa count of test rats and the control which were significantly significant (P<0.05). The reduction in spermatozoa count in rats exposed to dietary aflatoxin M1 from 280±24.35 in 2 weeks of rat’s dietary exposed to aflatoxin M1 to 206±14.5 in 4 weeks and 140.0±25.69 in 6 weeks as compared with the control group with 378.3±15.4 (x10⁶cells/mm³), thus suggest that aflatoxin M1 caused the destruction of sperm cells. This effect must have been achieved through several mechanism: Changes in the tonicity (osmolality) of the extracellular fluids which is as a result of acid-base imbalance; binding of aflatoxin M1 to sperm cells leading to active membrane damage and subsequent lysis of the sperm cell. All these factors may act independently or in concert to bring about a decrement in spermatozoa count. The observed decrease in spermatozoa count are in tandem with works on rats exposed to aflatoxin M1 [14, 35, 36, 45].

Abnormalities were observed when rats were exposed to aflatoxin M1 as reported in this study compared with the control group that had as high as 90.83±0.833 (%) normal sperm cell and only 9.71±0.833 (%) abnormal sperm cell. Some of the abnormalities as shown in the micrograph (Plate 1, 2, and 3) where, Headless spermatozoa, disjoined body, bent head, tailless sperm cell, double tailed sperm cells and short tailed sperm cells. There was a significant increase in abnormal sperm cell in week six with 35.83±3.745(%), 30.0±0.00 in week four and the lowest abnormalities in week two one (25.00±2.236(%), compared to the control group. As documented in previous studies which showed a 95±5

Table 1. Effect of Aflatoxin M1 on the sperm cell quality, comparing the control group and the treated group

Groups of Rats Fed on Aflatoxin M1 Diet (Weeks)	Total Sperm Cell Counts (Mean±S.E) N=6 (x10 ⁶ cells/mm ³)	Progressive Motility (Mean±S.E) N=6 (%)	Non Progressive Motility (Mean±S.E) N=6 (%)	Immotile (Mean±S.E) N=6 (%)
2	280.00± 24.35	71.67±3.07	11.67±1.66	16.67±2.10
4	206,67±14,53	55.00±9.91	11.67±1.66	35.00±11.4
6	140.00±25.92	37.50±9.97	10.83±2.00	53.33±12.2
Control	378.33±15.14	85.83±2.71	5.83±2.00	8.33±1.05

Table 2. Sperm Cell Morphology of Adult Male Wister Rats Fed with Aflatoxin M1 Diet

Groups of Rats Fed Aflatoxin Diet (Weeks)	Normal Morphology (Mean±S.E) N=6 (%)	Abnormal Morphology (Mean±S.E) N=6 (%)
2	75.00±2.23	25.00±2.23
4	70.00±0.00	30.00±0.00
6	64.17±3.74	35.83±3.74
Control	90.83±2.04	9.17±0.83

Plate 1-4: Sperm Cell Morphology Stained with the Improved Eosin and Leishman Stain (Ibeh *et al.*, 2018).



Plate 1: (i) Abnormal sperm cell, deformed head (ii) Headless spermatozoa



Plate 2: (i) Tailless sperm cell (ii) bent sperm cell

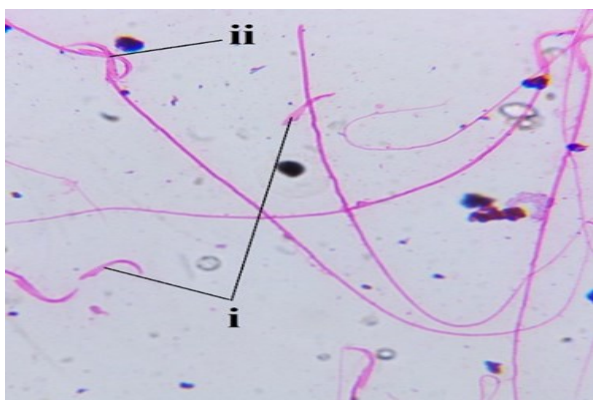


Plate 3: (i) Headless sperm cell (ii) Tailless sperm cell



Plate 4: (i) Bent head (deformed) (ii) Headless sperm cell (iii) Tailless sperm cell

(%) normal sperm cells in the control groups (Akbarsha *et al.*, 2007), which is also in tandem with this study. Abnormal sperm cells were significantly lower as observed in this study, compared to previous work that showed a higher percentage of abnormal sperm cells. This may be due to our short time of exposure of the rats to dietary aflatoxin as compared to other studies with a minimum of fifty-six days (56) exposure to aflatoxin M1 [15].

The increase in spermatozoa abnormalities may be directly related to the toxicity of aflatoxin M1 on the spermatocytes, which progresses to abnormal mitotic and meiotic division of the primary and secondary spermatocytes [16]. The basis of induced abnormalities can also be caused due to the phenolic ring structure of aflatoxin which induces mitochondria uncoupling and oxidative phosphorylation which eventually results in the swelling of the spermatid mitochondria [22, 23]. Leakages of cellular materials following the alterations of sperm membrane permeability may also contribute to spermatozoa abnormalities [37- 40].

Conclusion

The results of this study has revealed that aflatoxin M1 ingestion negatively affects sperm cell quality in male Wister rats. Sperm cell motility, count decreased and abnormalities increased with increased time of dietary exposure, from 2 to 6 weeks.

Conflict of Interest: There were no conflict of interest associated with this study

Acknowledgement: Immense thanks goes to the National Academy for the Advancement of Science, for their contribution in grants and in kind.

References

[1] Abdin MZ, Ahmad MM, Javed S. Advances in molecular detection of *Aspergillus*. Archives of microbiology. 2010;192(6):409-25

[2] Aksit S, Caglayan S, Yaprak I, Kansoy S. Aflatoxin: Is it neglected threat for Formula-fed infants? Acta Pediátrica Japónica.1997;39:34-36

[3] Ali N, Hashim NH, Yoshizawa T. Evaluation and

- application of a simple and Rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philipines. *Food Additives and Contaminants*. 1999;16:273-80
- [4] Arafa AS, Bloomer RJ, Wilson HR, Simpson CF, Harms RH. Susceptibility of various poultry species to dietary aflatoxin. *British Poultry Science*. 1981;22:431-6
- [5] Arangurén EM, Argüelles MJ. Detección de aflatoxina M1 en quesos frescos comercializados en el municipio de Yopal, Casnare, mediante la técnica de Elisa. Thesis, Pontificia Universidad Javeriana, Bogotá, Colombia. 2009;Pp225.
- [6] Asao T, Buchi G, Abdel-Kader MM, Chang SB, Wick EL, Wogan GN. Aflatoxins B and G. *Journal of American Chemical Society*. 1965;87:882-6
- [7] Castegnaro M, McGregor D. Carcinogenic risk assessment of mycotoxins. *Revue de Médecine Vétérinaire*. 1998;149:671- 8.
- [8] Codex Committe on Food Additives and Contaminants. Micotoxinas presentes en Alimentos Piensos. Observaciones sobre el proyecto de nivel máximo para la aflatoxina M1. En la leche Tema 16 del programa. 1999;13: 12-33
- [9] Codex Committe on Food Additives and Contaminants. La Comisión Europea; Seguridad Alimentaria. Observaciones de la Comunidad Europea para la Comisión del Codex Alimentarius, 24^o Reunión. 2-7 de Julio de 2001, Ginebra, Suiza. "Proyecto del nivel máximo para la aflatoxina M1 en la leche" (ALINORM01/12ApendiceX)". http://europa.eu.int/comm/foods/fs/ifsi/eupositins/cac/archives/cac_itm10a_es.html
- [10] Chen C, Pearson AM, Coleman TH, Gray JJ, Pestka JJ, Aust SD. Tissue deposition and clearance of aflatoxins from broiler chickens fed a contaminated diet. *Food and Chemical Toxicology*. 1984;22(6):447-51.
- [11] Chen SY, Chen CJ, Chou SR, Hsieh LL, Wang LY, Tsai WY, Ahsan H. et al., Association of aflatoxin B 1-albumin adduct levels with hepatitis B surface antigen status among adolescents in Taiwan. *Cancer Epidemiology, Biomarkers and Prevention*. 2001;10(11):1223-1226.
- [12] Diekman MA, Green ML. Mycotoxins and reproduction in domestic livestock. *Journal of Animal Science*. 1992;70(5):1615-27
- [13] Doherty WP, Campbell, TC. Aflatoxin Inhibition of rat liver mitochondria. *Chemical Biological Interaction*. 1973;7: 559-62
- [14] Hendrickse RG. Clinical implications of food contaminated by aflatoxins. *Annals Academy of Medicine Singapore*. 1991;20:84-90
- [15] Henry SH, Bosch FX, Bowers JC. Aflatoxin, hepatitis and worldwide liver cancer risks. *Advances in Experimental Medicine and Biology*. 2002;504:229-33
- [16] Herzallah SM. Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry*. 2009;114(3):1141-46
- [17] Jarvis BB. Chemistry and toxicology of molds isolated from water-damaged buildings, Mycotoxins and Food Safety. *Advances in Experimental Medicine and Biology*. 2002;504:43-52
- [18] Johnson DN, Egner PA, O'Brien G, Glassbrook N, Roebuck BD, Sutter T, Payne GA, Kensler, TW, Groopman JD. Quantification of Urinary Aflatoxin B1 Dialdehyde Metabolites Formed by Aflatoxin Aldehyde Reductase Using Isotope Dilution Tandem Mass Spectrometry. *Chemical Research in Toxicology*. 2008;21:752-60
- [19] Dvorackova I. Aflatoxin inhalation and alveolar cell carcinoma. *Case Report. British Medical Journal*. 1976;8:691-9
- [20] Fandohan P, Gnonlonfin B, Hell K, Marasas WF, Wingfield MJ. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in pre-harvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology*. 2005;99(2):173-83
- [21] Fandohan P, Zoumenou D, Hounhouigan DJ, Marasas WF, Wingfield MJ, Hell, K. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology*. 2005;98(3):249-59
- [22] Hesseltine CW. Conditions Leading to Mycotoxin Contamination of Foods Feeds. In: *Mycotoxins, Other Fungal Related Food Problems*. Joseph V. Rodricks (Ed), American Chemical Society. 1976;Pp.1-22.
- [23] International Agency for Research on Cancer. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation. *IARC Monographs on the evaluation of the carcinogenic risk to humans*. International Agency for Research on Cancer. 2002;Pp.450
- [24] Kamdem LK, Flockhart DA, Desta Z. Dominant contribution of P450 3A4 to the hepatic carcinogenic activation of aflatoxin B1. *Chemical Research in Toxicology*. 2006;19:577-86

- [25] Food and Agricultural Organization. United Nations Food and Nutrition Paper 64. Worldwide Regulations for Mycotoxins..1997;Pp.1056
- [26] Food and Drug Administration, USA. Action levels for added poisonous or deleterious substances in food. Notice Federal Register. 1998;53:5043-4
- [27] Frisvad JC, Smedsgaard J, Larsen TO, Samson RA. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Studies in Mycology*. 2004;49:201-42
- [28] Gimeno A. Aflatoxina M1 no leite. Riscospara a saúde pública, prevenção e controlo. *Alimentação Animal (Revista de la Associação Portuguesa dos Industriais de Alimentos Compostospara Animais*. 2004;49:32-44.
- [29] Greene HJ, Oehme FW. A possible case of equine aflatoxicosis. *Clinical Toxicology*. 1976;9:251-4
- [30] Groopman JD, Wogan GN, Roebuck BD, Kensler TW. Molecular biomarkers for aflatoxins and their application to human cancer prevention. *Cancer Research*. 1994;54,(7)1907s-11.
- [31] Groopman JD, Kensler TW, Wild CP. Protective Interventions to Prevent Aflatoxin-Induced Carcinogenesis in Developing Countries. *Annual Review of Public Health*. 2008;29(1):187-203
- [32] Guengerich FP, Johnsen WW, Ueng YF, Yamazaki H, Shimada T. Involvement of cytochrome P450, glutathione S-transferase and epoxide hydrolase in the metabolism of aflatoxin B1 and relevance to risk of human liver cancer. *Environmental Health Perspectives*. 1996;104: 557-62
- [33] Hall AJ, Wild CP. Epidemiology of Aflatoxin-Related Disease. In: *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. Eaton DL and Groopman JD eds. San Diego, CA, Academic Press, Inc. 1994; 233-58
- [34] Hafez AH, Megalla SE, Mohamed AA. Effect of dietary aflatoxin on the morphology of buffalo bull spermatozoa. *Mycopathologia*. 1982;77:141-4
- [35] Hafez AH, Comma A, Mousa SA, Megalla SE. Aflatoxin and aflatoxicosis IV. The effect of dietary aflatoxins on adult fertile male and female rabbits at various reproductive conditions. *Mycopathologia*. 1983;83:183-6
- [36] Helferich WG, Baldwin RL, Hsieh DPH. Aflatoxin B1 Metabolism in Lactating Goats and Rats. *Journal of Animal Science*. 1989;62:697-705
- [37] Ibeh IN, Uriah N, Ogonor UI. Dietary exposure to Aflatoxin in human male infertility in Benin city, Nigeria. *International Journal of fertility and menopausal studies*. 1991;39:208-14
- [38] Ibeh IN, Uriah N, Ogonor UI. Dietary exposure to Aflatoxin in Benin City, Nigeria. a possible public health concern. *International Journal of food microbiology*. 1994;14:171-4
- [39] Ibeh NI, Owie IC, Omorodion NT. Effect of aflatoxin B1 dietary exposed Wister rats; spermatogenesis, dynamics, kinetics and loss of rat epididymal sperm proteins. *International Journal of Research in Health Sciences*. 2017;5(2):21-32
- [40] Ibeh, NI, Otabor FE, Oyedeji JT, Emili A, Omorodion NT. Improvised Eosin and Leishman as a morphological stain for sperm cell analysis, Adult wister rats and rabbits as model of study. *Acta scientifica Microbiology*.2018;1(1):18-20
- [41] Kamimura H. Removal of mycotoxins during food processing. *Mycotoxins and Phycotoxins Elsevier*. 1989;169-76
- [42] Kalorey DR, Daginwata HF, Ganorkar AG, Mang KN. Serum zinc and iron status in experimental aflatoxin in chicks. *India Journal of Veterinary Research*.1996;5:28-32
- [43] Ketterer PJ, Blaney BJ, Moore CJ, McInnes IS, Cook PW. Field cases of aflatoxicosis in pigs. *Australian Veterinary Journal*. 1982;59(4):113-7
- [44] Klein PJ, VanVleet TR, Hall JO, Coulombe RA. Dietary Butylated Hydroxytoluene Protects against Aflatoxicosis in Turkeys. *Toxicology and Applied Pharmacology*. 2002;182(1):11-9
- [45] Hendrickse, R. (1999). Of sickturkeys, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: Research on the influence of aflatoxins on child health in the tropics. *Annals of Tropical Pediatrics*. 19:229-36.