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Effect of Aflatoxin Laced Diet on the Utero-Fallopian Tube Histology in New Zealand White Rabbits

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Abstract

Fungi are extremely adaptable organisms with the capacity to metabolize a large variety of substrates over a wide range of environmental conditions and are produced only under aerobic conditions. Aflatoxin contamination is promoted by stress or damage to crops due to drought prior to harvest and inadequate drying during storage. High concentrations of aflatoxins above acceptable levels are in some instances found mainly in feed grains particularly maize and groundnuts in the tropics. Aflatoxins are secondary metabolites that pose serious hazards to animal and human beings. Their severity and effect of poisoning depends on age, where younger animals are more prone than mature ones. Different sexes of particular species of animals, duration of exposure and the amount of aflatoxin being consumed cause different effects. The experimental rabbits were kept inside a housing structure which had wide windows closed with a combination of welded and chicken wire mesh to ensure free air circulation, but protected from entry of birds and predators. The house was reinforced against rodents, though rodenticides were used to check any possible presence of rats. The rabbit house was well ventilated, with sufficient light through translucent iron sheets at the roof and wide windows to ensure 12-hour light with a room temperature of 18 – 22 º C. One rabbit was picked at random from each of the four treatments to be taken to the laboratory to harvest the uterus and fallopian tubes to determine their effects following intake of aflatoxin laced feeds. At the laboratory, the rabbits were kept in air tight glass cages for 30 minutes with a piece of cotton wool that was socked with 37% formalin inside to bring them into unconscious state, meant to facilitate humane sacrificing for purposes of harvesting the structural tissues, which were there after preserved in 37% formalin solution before sectioning it for microscopy. The abdomen was opened and the uterus and fallopian tubes harvested for preparation of histological sections in readiness for microscopic examination. The organs were treated with a fixative at the ratio of 2:1 for formalin and tissue at room temperature and allowed to dry for 12 hours. The tissues were fixed, embedded in paraffin wax, sectioned, stained using hematoxylin and eosin, put onto the slides and examined using the microscope. The features of the cells in treatments 1 and 2 as observed appeared productive as indication of the uterus' proliferative stage. There was mild focal aggregation of acute inflammatory neutrophil cells in the lamina propria in the submucosa of the endometrium of the uterus observed in treatment 2. The uteri in treatments 3 and 4 revealed marked necrosis of the lining of both the epithelium and the endometrium. Degeneration of the lining of epithelium of the uterine

glands, appearing as vacuoles with mild leucocytic cell infiltration into the submucosa and lamina propria and focal necrosis of lamina epithelialis were observed. Examination of the cross-section of the fallopian tubes showed minor effects in treatments 3 and 4. These defects manifested as vacuolization of the lining of the epithelium of the endometrium. In conclusion, aflatoxin inclusion in New Zealand white rabbit diets at levels above 100 ppb has serious defects on both the uterus and fallopian tube to the extent that it may affect the reproductive function of the rabbits.

Keyword: Aflatoxin, fallopian tube, Histology, uterus

INTRODUCTION

Mycotoxins are produced in temperate, sub – tropical and tropical climates whenever rainfall and humidity are experienced during the grain harvesting season (WHO, 2005). Fungi are extremely adaptable organisms with the capacity to metabolize a large variety of substrates over a wide range of environmental conditions and are produced only under aerobic space (Cardwell & Cotty, 2002). Aflatoxin contamination is promoted by stress or damage to crops due to drought prior to harvest and inadequate drying during storage (Sasaki *et al*., 2002; Turner *et al*., 2005). High concentrations of aflatoxins above acceptable levels are in some instances found mainly in feed grains especially maize and groundnuts in the tropics (Kitya *et al*., 2009). They are secondary metabolites that pose serious hazards to both animals and human beings (Rustemeyer *et al*., 2010; Jamal *et al*., 2012). The severity and effect of poisoning depends on age, where younger animals are more prone than mature ones; while different sexes of particular species of animals are affected differently, the amount of aflatoxin being consumed and duration of exposure manifest different effects too (Gugnani, 2000).

Aflatoxin in sera ingested through food or feed causes harmful changes in animal tissues as reported by WHO (2018). High levels of exposure is fatal to mammals, birds, fish as well as human beings. The liver is the principal target organ affected, however high levels have been found in the kidneys, lungs and the heart of individuals that died of acute aflatoxicosis (Sun *et al*., 2015).

Rabbit litter size has been shown to have declined in the recent past from a high of 7 – 10 Kittens to 3 – 6 over time and their conception rates have declined, leading to increased generation interval (Mailafia *et al*., 2010). Aflatoxin could be one of the causes of this low litter size by interfering with hormone secretion, damage to spermatozoa or organs that play important roles in reproduction. Decline in reproduction among domestic animals reared intensively has been observed by stock owners and the current study seeks to establish whether there exists links between such declines in performance to aflatoxin. It is necessary to establish the effects of aflatoxin on organs that play important roles in secretion of reproductive hormones and other physical actions so that corrective and preventive measures can be put in place by producers and other stakeholders. Fertility is an important parameter of productivity because in some animal species, the number of offspring's born per parturition and the regularity are an indicator of high productivity (Petrovic, 2000). Animal fertility and the factors that influence it, along with the liter size are matters of concern to producers. Reproductive life starting from the onset of puberty, fertility rating, and twinning rate are factors that contribute to the fertility of an animal (Robson & Smith 2011). The uterine environmental health plays an important role in reproduction. The hormones oestradiol and progesterone prepare a healthy uterus for the attachment and maintenance of pregnancy (Hazlerigg & Simonneaux 2015).

The posterior pituitary gland secretes oxytocin hormone that is responsible for healthy uterine contractions also during birth. The uterine muscles are not responsive to oxytocin hormone until late during pregnancy, this is the moment oxytocin receptors in the uterus will have reached the peak, the tissues in the uterus and cervix are stretched and this stimulates oxytocin discharge during birth. The intensity of the contractions increases when the blood levels of oxytocin peak via a positive feedback mechanism until when the birth process is completed (Petrulis 2013).

The reproductive activities of the female of any animal species are the first to be arrested whenever the animal is confronted by debilitating illness, nutritional deficiencies or life-threatening diseases. Likewise, conception is only the beginning of reproduction for the female, as it must go through gestation and the birth of a healthy offspring. The uterine environment therefore, must be healthy for gamete encounter and fertilization, embryonic development, attachment, gestation and successful completion of pregnancy (Pineda 1989). The objective of the current study was to determine the histopathology of the uterus and fallopian tube of female New Zealand white rabbits fed on diets laced with aflatoxin.

MATERIALS AND METHODS

Study Site

The current study was carried out at the University of Eldoret, within the coordinates 0°31'N (Latitude), 35°16'E (Longitude) and at an altitude of 2100 meters above sea level. The location has an average annual temperature of 17°C and a bimodal annual rainfall of 1100mm. The study was carried out in a house with room temperature range of 18 - 22ºC and a relative humidity range of 53 - 60%. The light in the experimental house was provided for 12 hours to facilitate feeding, watering and observations of rabbits at any time of the day. The house allowed minimum air movement (wind) and was restricted from human access to minimize any possible rabbit disturbance and the risk of inhalation of aflatoxin by any human visitor.

Experimental rabbits

The New Zealand white breed (*Oryctolagus cuniculis*) of female rabbits used for the study were acquired from Tatton farm of Egerton University demonstration and research unit in Nakuru County, who kept the breed under confinement and fed on commercial concentrate. The farm produced rabbits for research, teaching and supply to both farmers and other practitioners. The rabbits were familiar with cage rearing and consumption of concentrate mash and/or pelleted diets. Upon arrival to the experimental site, the rabbits were each assigned a number and their ears notched.

Housing and care of experimental rabbits

The experimental rabbits were kept inside the housing structure which had wide windows closed with a combination of welded and chicken wire mesh to ensure free air circulation, but protected from entry of birds and predators. The house was reinforced against rodents, though rodenticides were used to check any possible presence of rats. The rabbit house was well ventilated, with sufficient light through translucent iron sheets at the roof and wide windows to ensure a 12-hour light with a room temperature of 18 – 22 ºC. The house was thoroughly cleaned, disinfected and anti-mite dust applied to keep off any possibility of the entry of mites in the house. Saw dust was spread on the floor, under the rabbit cages before disinfection. The saw dust was removed after a week to ensure the least rotting which could easily have caused some infection to the rabbits.

Rabbits for each treatment were kept in cages built to measure 80 X 50 X 30 cm. These rabbit cages were built using timber frames whose sizes measured 3"x 2'', with welded wire mesh on the sides and the floor. Both the side and floor welded wire mesh were reinforced with chicken wire so that the rabbit feet didn't pass through, but also the rabbits walked on the floor without injury or the feet getting through.

Feeding of the experimental Rabbits

The persons attending to the experimental rabbits were dressed on protective clothing that included laboratory coats, gumboots and aspirators at any time while working to minimize possible entry of aflatoxin into their bodies, which has been reported to enter through wounds or nostrils by inhalation. Each treatment group of rabbits were given 200 gm of feed twice daily; at 8.00 am and 3.00 pm using stable broad-based earthen pots as feed troughs, to ensure they don't topple over incase the rabbits stepped on them. Feeds were weighed using an electronic balance before it was provided in the feed trough. Feeding was carried out twice daily to ensure that the troughs were not filled to the brim for purposes of minimizing spillage and to avail feed to the rabbits every time of the day, as well as to ensure a stable continuous nutrient supply for body metabolic functions.

A total of sixteen rabbits of three months of age were randomly distributed to four treatments using completely randomized design procedure. The experiment used 4 rabbits in each treatment in a repeated measurement study. The rabbits were allowed seven days to familiarize with the diet, the weighing, the healing of ear notch and the new groupings in the cages.

Harvesting and Preparation of rabbit organs

One rabbit was picked at random from each of the four treatments for examination of the uterus and fallopian tube to determine the histological alterations following intake of aflatoxin laced feeds. The rabbits were kept in air tight glass cages with a piece of cotton wool that was socked with 37% formalin for 30 minutes to bring them into unconscious state meant to facilitate humane sacrificing of the rabbits. The abdomen was opened and the uterus and fallopian tube harvested and there after preserved in 37% formalin solution. Tissue preparation for histological work was carried out in the laboratory according to procedures given by Abert et al. (2015) which included fixation of cells and tissues using formalin, embedding using paraffin wax and sectioning using the microtome. The sectioned tissues were stained using hematoxylin and eosin, which are the most common stain combinations for histological examination.

The histological appearances of cells of the uterus and fallopian tubes were examined using a binocular microscope (Olympus CX2 Model, Japan) at magnification X40. The microscope was fitted with a digital camera that was used to take micrographs of the cells.

RESULTS

The uterus from treatments 1 showed anatomically normal histological structure with minimum aflatoxin effects. There was mild focal aggregation of acute inflammatory neutrophil cells in the lamina propria in the submucosa of the endometrium, observed in treatment 2. The features of the cells in treatments 1 and 2 as observed appeared productive as indication of the uterus' proliferative stage.

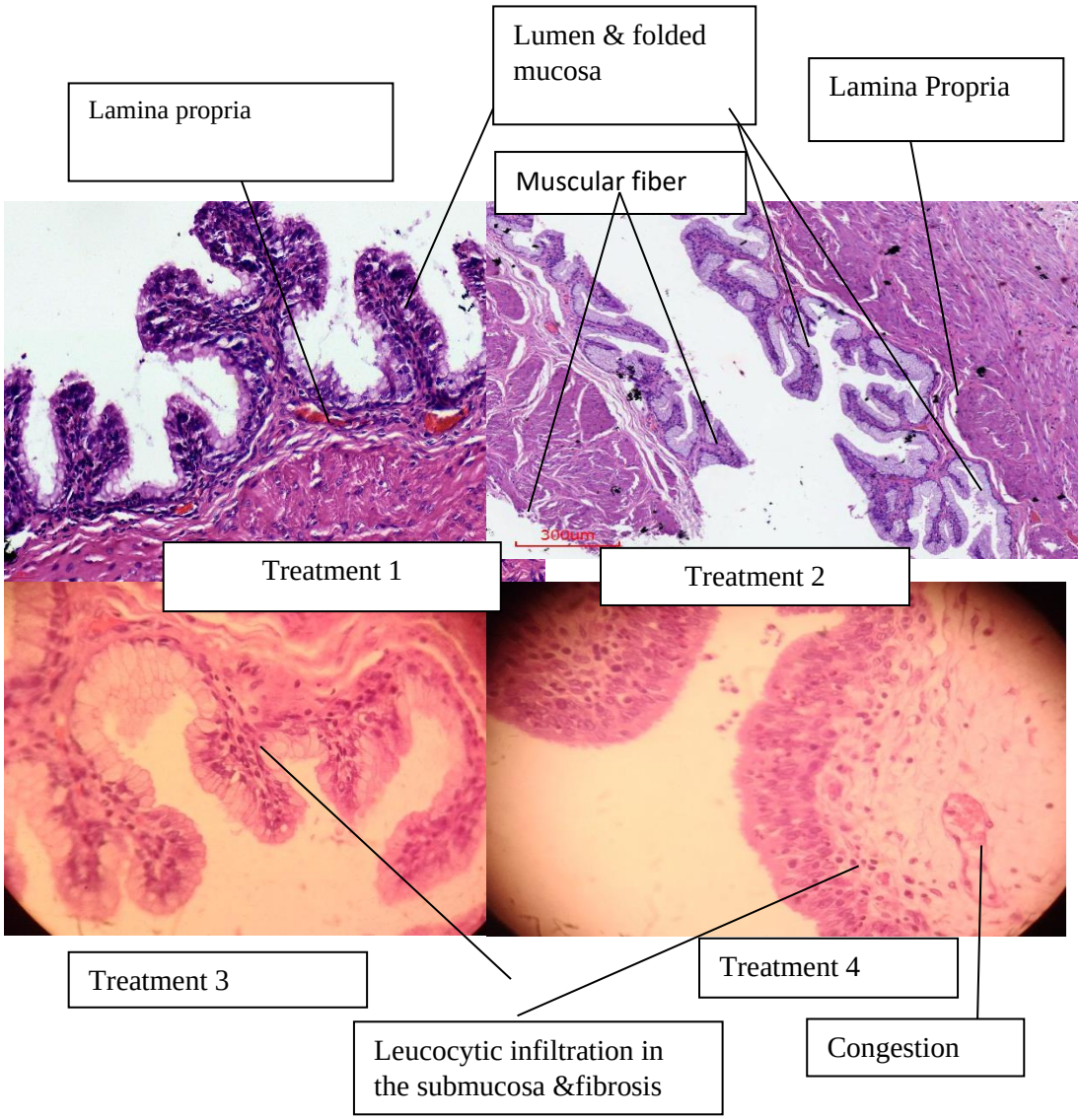

Figure 1: The micrograph showing the cross section of the uterus

The uteri in treatments 3 and 4 revealed marked necrosis of the lining of both the epithelium and the endometrium. Degeneration of the lining of epithelium of the uterine glands, appearing as vacuoles with mild leucocytic cell infiltration into the submucosa and lamina propria, and focal necrosis of lamina epithelialis were observed. Congestion of the myometrial blood vessels and massive fibrosis of the lamina probria were noticed in some instances.

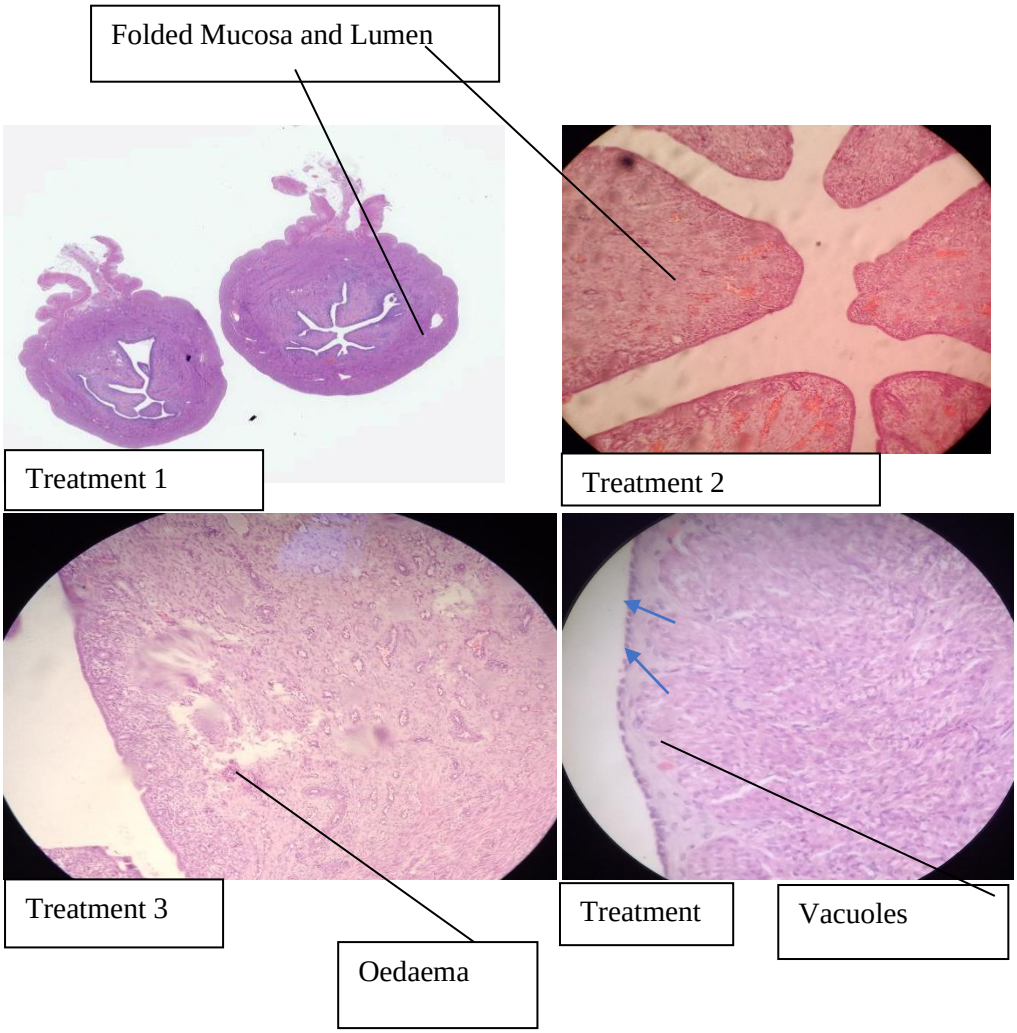

Figure 2: The micrograph of fallopian tube indicating folds and vacuoles. The arrows (⟶) indicate the vacuoles at the lining of the epithelium.

Examination of the cross-section of the fallopian tubes given in figure 2 Showed minor effects in treatments 3 and 4. These defects manifested as vacuolization of the lining of the epithelium of the endometrium.

DISCUSSION

The Uterus is important as it serves as a tubular structure for the transportation of spermatozoa into the oviduct where fertilization takes place and latter receives the embryo for implantation to the uterine wall to begin the gestation period. It is a vital structure of reproduction where the conceptus is implanted, nourished and develops into a foetus during gestation. Any damage to the structure has the potential to impair normal uterine function, and may result in abortion or embryonic death culminating in failed reproduction of the animal or low birth weights of the offspring (Shaw *et al*., 2010). The implantation process requires primarily, the support of sufficient quantities of oestrogen and progesterone hormones. Although other hormones may be at play in the control of a host of cells to carry out the implantation, growth and development process of the foetus (Shaw *et al*., 2010).

Uterine structures of the female rabbits in treatments with aflatoxin in the current study showed marked histopathological changes manifested as necrosis of the epithelium of the endometrium. These showed greater damage compared to what Abd El-Wahab (1996) observed as ulceration when he fed 0.15 mg afB1/ kg body weight to rats. The same researcher reported degenerative changes in the uterine gland at much higher aflatoxin dose of 7.0 mg / kg body weight. The endometrium is the most specialized, active portion of the uterus that gives responses to hormonal signals and initiates actions that lead to prolactin production in non-pregnant animals. Both oestradiol and progesterone are responsible for preparing the endometrium for possible implantation of the foetus incase conception has taken place (Shaw *et al*., 2010).

The reason for the differences in the intensity of damage could be attributed to different animal species and the family or species of cytochrome P450 enzymes that metabolized the aflatoxin in the liver, the length of time of exposure and the quantity of aflatoxin. Other reports show reduction in uterine sizes, increased foetal resorption and uterine death among some animal species, including rats, when exposed to high doses of aflatoxin (Gupta 2012). The uterus is known to put up defensive actions for survival and recover through the cell infiltration observed in the current study in the submucosa of the endometrium and may include the action of machrophages and T cells in an effort to wade off hypersensitivity to aflatoxin.

There were some effects at the stroma portion of the fallopian tube, which could be associated with invaded cells due to the effects of aflatoxin. Vacuolization at the stroma-edge of the fallopian tube just after the cilia, affects fat components of the cell, this portion is associated with the synthesis of hormones for endocrine function.

The fallopian tubes are located at either side of the abdomen and they are also referred to as oviduct and are funnel tube-like structures, which serve as channels for the transportation of ovum from the ovary to the magnum, where they are fertilized before the embryo is moved by the propping action of oxytoxin hormone on smooth muscles and moved to the uterus for implantation. Vacuolization refers to a change in morphology of animal cells following an infection by either bacteria, virus or contamination with substances of low molecular weight such as exudates from molds or those which are chemical in nature. Reports indicate the involvement of septin in processes associated with intracellular vacuolization in membrane related phagosomes (Hacker 2018). Membrane vacuole development appears to be linked to any infection. The vacuolization of the epithelium and the endometrium caused by aflatoxin consumption in the current study, risked the functioning of the smooth muscles and the cilia that facilitate the transportation of the ovum from the ovary, whereby, it may hasten its movement due to the possible inflamed nature of the epithelium or cause a functional cessation. The ciliate function is controlled by oestrogen and progesterone hormones under normal functional status of the animal, and they initiate ova mobility under the regulation of cilia and supported by the peristaltic action of a rhythmic contraction of the fallopian tube smooth muscles (Nutu *et al*., 2009). Low levels of oestrogen in plasma could also hinder the working of the cilia while Prostaglandins have likewise been found to upgrade the contractility of smooth muscles, thus facilitating the beating action of cilia and eventual transportation of ovum (Waggren *et al*., 2008). Reproductive function of the rabbit can be fully running if both the physical and endocrinological health are guaranteed through the consumption of aflatoxin free-nutrient balanced diets.

CONCLUSION AND RECOMMENDATIONS

Aflatoxin of more than 100 ppb in New Zealand white diets have deleterious effects on the uterus and fallopian tube. These effects can affect the reproductive functions of these structures, which may cause the rabbit to have small litter sizes or render them infertile.

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