Effect of Aflatoxin on in vivo Nutrient Digestibility and Kidney Integrity in Newzealand White Rabbits

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ABSTRACT

Contamination of crops by mycotoxin, particularly those produced by Aspergillus fungi, occurs at any stage of food production. They infect crops from pre-harvest to storage under favourable temperature and humidity, in similar conditions prevailing at the tropical region. Contamination also occurs due to dumping of food products, introduction of contaminated commodities into food chain during chronic food shortage due to drought, war, political and economic instability. Aflatoxin affects animals of all species and cause harm to most organs, and has been declared as carcinogenic, particularly in the chronic form. The objective of the current study was to determine the digestibility and kidney damage when feed containing moderate level of the toxin are fed to rabbits. The study used four rabbits in each of the four treatment diets containing O, 100, 200 and 400 ppb of aflatoxin. Feed intake and faecal output were determined every week and a sample taken to the laboratory for nutrient level determination and the digestibility worked out. Two rabbits were humanely sacrificed at the end of the study, their kidneys removed and a smear prepared on slides. The slides were observed at a magnification of x 40 using an electronic microscope. Nutrient digestibilities were: 78.4, 75.3, 72.8 and 71.2; 70.9, 70.8, 69.4 and 66.6, 25.9, 21.2, 22.7 and 23.0 for dry matter, organic matter and neutral detergent fibre respectively. There was significantly low digestibility for treatment 4 for dry matter and organic matter but there were no treatment difference for crude protein acid detergent fibre and ash. Treatments with aflatoxin showed pathological effects on the kidneys.

Key words: In vivo digestibility, aflatoxin, kidney

INTRODUCTION

Contamination of crops by mycotoxin, particularly those produced by Aspergillus fungi and the level of contamination occurs at any stage of food production from pre-harvest to storage. They occur in the mycelium of filamentous fungi but may be present in the spore. The factors influencing their production include; favourable temperature range of 24 to 35^{o}C and relative humidity of 7 to 10%, which in most cases is rampant within tropical regions (Williams *et al*., 2004). Mycotoxins have been reported to contaminate about 25% of the world's food supply each year, particularly cereals and groundnuts that are produced for consumption and approximately 4.5 billion people are exposed worldwide (Williams *et al*., 2004).

Among the various types, aflatoxin is the most common type in the tropical and sub-tropical regions where it is an important public health problem in underdeveloped countries that have limited or undeveloped modern agricultural and medical technologies for their detection and control. Aflatoxins are poisonous products of moulds or non-mould fungus found in grains, grass hay or legumes harvested and stored under moist conditions. Some feeds may be more prone to contamination, particularly grains, grain by–products, protein concentrates, finished feeds, oilseeds and forages. The severity of aflatoxin contamination is influenced by environmental factors such as excessive moisture (both when the crop is in the field and in storage), temperature extremes, humidity and variations in harvesting practices (Walder *et al.*, 2010).

The presence of aflatoxin in food is often overlooked due to public ignorance concerning their existence, lack of sufficient regulatory mechanisms, dumping of food products and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, war, political and economic instability (Muthomi *et al*.,2009). High concentrations of aflatoxins above acceptable levels are in some instances found in stable food grains that include maize, nuts and other cereals in developing tropical nations (Kitya *et al*., 2009).

Mammalian animal species contain two bean-like kidneys on either side of the body which serve the primary purpose of removing waste products of muscle, cell breakdown and food. After food is absorbed and metabolized, the body uses what it requires and the rest are send to the blood stream from where it will pass through the kidneys for filtering by the millions of nephrons. The kidney contains nephrons, which does the filtration, and then the network of capillaries takes both the waste (mainly products of metabolism that include urea, creatinine and proteins) and water into the tubule, from where it will be transported to the urethra and finally the bladder for deposal. The kidney also regulates the osmotic pressure through the balance of ions in the body using sodium, phosphorus with the aid of mineralcorticoid hormone (http://www.webmed.com/a-to-z guides/function-kidney).

Kidneys, also produce hormones, whose role in life process cannot be overlooked. They include erythropoietin, which stimulates the manufacture of red blood cells, rennin; regulates blood pressure and the active form of

vitamin D, which maintains Calcium level for borne formation and nerve transmission in the animal's body (htt://www.webmd.com/a-to-z guides/function-kidney).

Chemical composition of a feed affects its digestibility through different reactions by chemical entities, where some may diminish the opportunity for digestive enzymes to act on substrates (Khan *et al*., 2003). Fermentation of the feed composition may modify the gastrointestinal environment and result into depressed or enhanced digestibility of the diet. Diets with aflatoxin may interfere with digestion depending on its effects on enzymes and microbes in case of ruminants. The objective of the study was to determine effects of aflatoxin levels on *In vivo* nutrient digestibility and kidney pathology of Newzealand white female rabbits.

MATERIALS AND METHODS

Study site

The current study was carried out at Eldoret, Uasin Gishu County, located within the coordinates 0°31'N (Latitude), 35°16'E (Longitude) and at altitude range of 2000- 2095 meters above sea level. The area has an annual average temperature of 16.6°C and rainfall of 1103mm.

Experimental animals and Housing

Four Newzealand white ***(****Oryctolagus cuniculus)* breed of female rabbits were used for each treatment in cages measuring 80 x 50 x 30 cm and fitted with feeding space that was enough for feed requirements for the day, while water was availed *ad libitum*. The rabbit cages were well built inside a ventilated house, provided with sufficient light and protected against wind, direct sun rays and rainfall. The housing was also used to protect the rabbits from predators and thieves.

Feed concentrates were compounded by Chania feed millers with a balance of nutrients for growing rabbits, but free from aflatoxin binders. Then Everest Store and Lab Technologies were contracted to incorporate the aflatoxin in the quantities recommended for each treatment. After the diets were supplied, a sample of each was taken for laboratory analysis to cross check and ascertain the nutrient and aflatoxin levels.

Diets and Design

The study ran for 45 days during which time the rabbits were fed on treatment diets. A total of sixteen rabbits of two and a half to three months of age were distributed to four treatments using simple random procedure. They were given seven days of acclimatization to the diet, the numbering procedures and new groups in the cages. The study treatments included; 1–control (concentrates without aflatoxin), treatment 2– concentrates containing 100 ppb of aflatoxin, treatment 3- concentrates containing 200 ppb of aflatoxin and treatment 4- concentrates containing 400 ppb of aflatoxin in feed dry matter.

Analyses

Feed and faecal samples were analyzed for nutrient contents according the methods of AOAC (2000). The nutrient digestibility was worked out using the formula provided by Khan *et al*., (2003);

$$\text{Digestibility (\%)} = \frac{\text{Nutrient in feed - Nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

The data for nutrient digestibility was subjected to analysis of variance.

At the end of the study, two rabbits per treatment were humanely sacrificed and their kidney harvested. A sample was removed and smears prepared for pathological examination using electronic microscope. The appearance of organs was determined and represented in pictorial form. Significant means of the nutrient digestibility data were separated using tukeys test.

RESULTS

The *In vivo* apparent digestibility of nutrients in diets fed to rabbits in each treatment showed significant difference as provided in table 4.2.

Table 1.The *In vivo* apparent nutrient digestibility (%) of diets fed to rabbits in each treatment

Treatment	Dry Matter	Organic Matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Ash
1	78.4[a]	70.9[a]	12.9	25.9[a]	4.2	11.5
2	75.3[b]	70.8[a]	11.6	21.2[b]	12.2	4.5
3	72.8[c]	69.4[a]	13.2	22.7[b]	4.2	3.4
4	71.2[d]	66.6[b]	13.3	23.0[b]	5.5	4.6
sem	1.4	0.4	0.56	1.6	2.1	2.2
	**	**	NS	**	NS	NS

As shown in table 1, the digestibility of dry matter (DM) and organic matter (OM) decreased with increasing aflatoxin content in the diet. The digestibility of crude proteins (CP) was not significant ($P \geq 0.05$) among treatments but showed an insignificant increase in trend with increasing levels of aflatoxin. Neutral detergent

fibre (NDF) digestibility was significantly (P≤0.01) low for treatments with aflatoxin. There was no clear trend on the digestibility of acid detergent fibre (ADF) and Ash in relation to aflatoxin levels contained in the treatment diets.

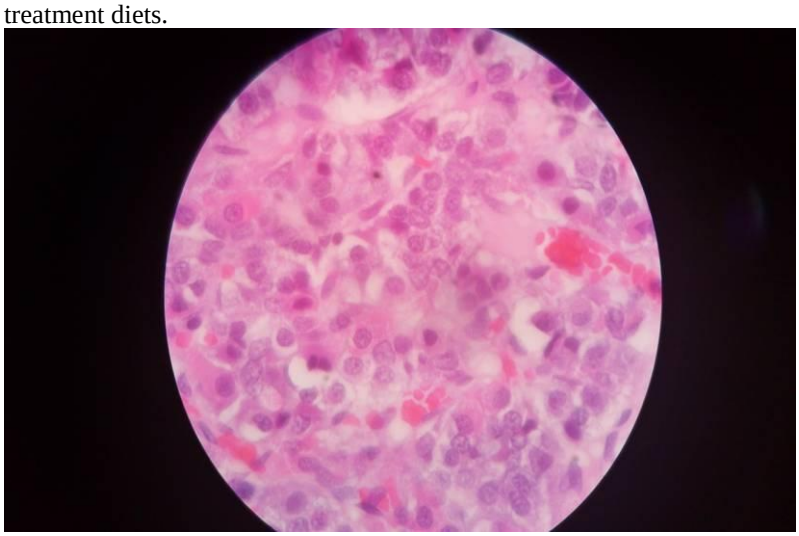

Figure 1: Normal kidneys with glomerulii cells in treatment 1

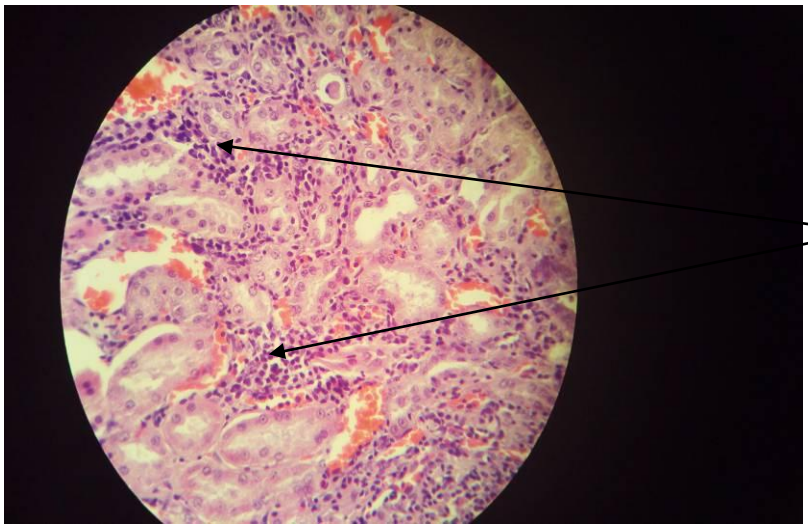

Figure 2: Kidney with dark areas indicative of inflammation/ necrosis in treatment 2.
Focal inflammatory areas are visible. These are interstitial and peritubular cells both in the cortex and medulla appearing inflamed

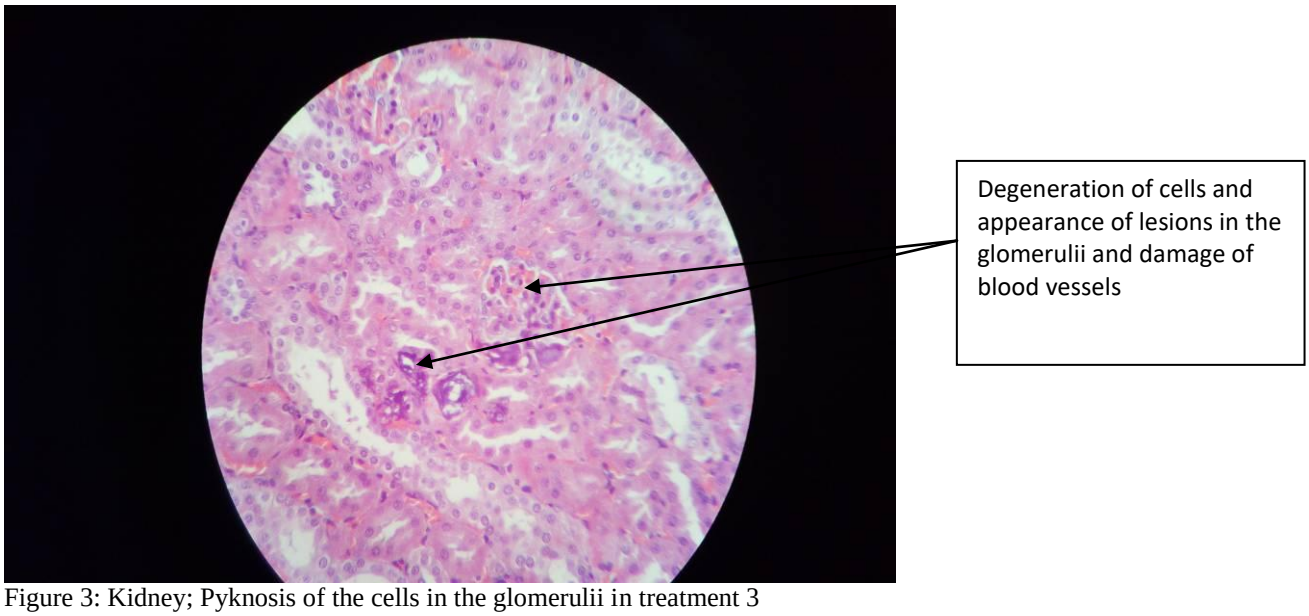

Figure 3: Kidney; Pyknosis of the cells in the glomerulii in treatment 3

The Presence of dark cells was indicative of the degeneration of the organ. Damage and lesions in blood vessels and glomerulus cells was an indicator of death, while other cells of the kidneys appeared normal.

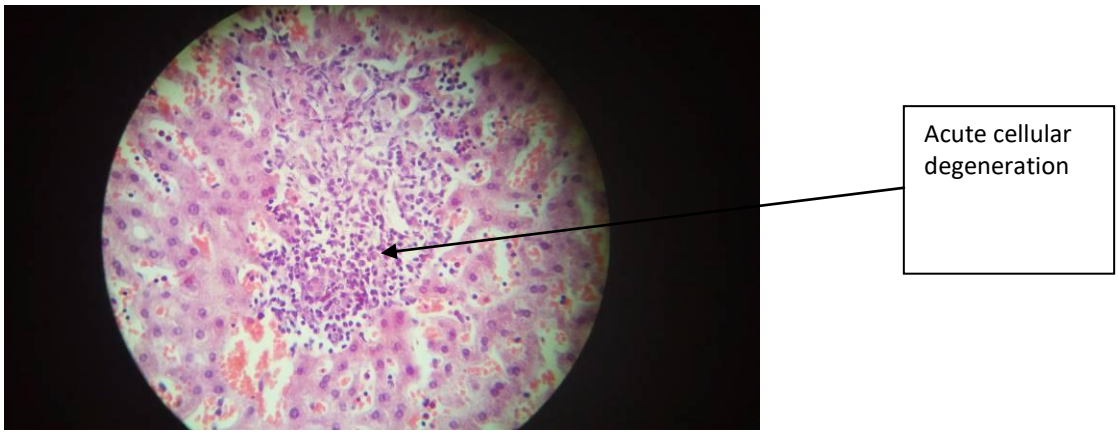

Figure 4: Kidney in treatment 4 showing cells appearing inflamed.

The glomerulus of the kidneys shown in figure 4 was quite affected by the toxin. It is an indication of acute cellular degeneration of the tubules.

DISCUSSION

Most animals of all species are susceptible to acute toxic effects of aflatoxin, but mature individuals have higher tolerance than young stock (Williams *et al*., 2004) of the same species. Rabbits are sensitive to aflatoxin with low LD_{50} of 0.3 mg/kg body weight for young growers (Clark *et al*., 1982; Busby & Wogan, 1979). The clinical signs and clinicopathological changes caused by aflatoxin in rabbits have been reported to be similar to those of swine, goats and cattle, therefore is the potential model for developing technologies for these species (Carnaghan *et al*.,1967).

The first interaction between the rabbits and aflatoxin occurred at the gastrointestinal tract, where its integrity was necessary to guarantee the animal's nutritional welfare. The gastrointestinal tract is an area of high protein turnover at the gut epithelium. Whenever the integrity of the intestinal mucosa is affected, nutrient absorption decreases (Grenier & Applegate, 2013). The *In vivo* apparent digestibility of nutrients in the current study manifested a declining trend from the control to the treatments with the highest aflatoxin in the diet, particularly for dry matter, organic matter and neutral detergent fibre. This was similar to the findings of other authors (Marai & Asker, 2008; Sheheta, 2002) who reported significant decrease in dry matter and Nitrogen free extract digestibility when rabbits were fed diets containing aflatoxin. Aflatoxin causes nutritional interference in animals but the threshold of this act is yet to be established. The efficiency of feed utilization has been reported to be lower by 7-10% for exposed pigs and poultry (Shane, 1993). The current study found the ballooning of both the small and large intestines, and peeling of the epithelial mucosa, which is thought to interfere with digestion of nutrients from the diet of the consuming animal.

Grenier and Applegate (2013) reported modulation of digestive enzyme activity by aflatoxin consumption. Such disturbances may lead to gastrointestinal disorder, which affect digestion, nutrient uptake and animal reproduction. This interference could be responsible for the unpredictable apparent digestibility of proteins due to varied amount of endogenous proteins. Toxicity of dietary aflatoxin in mammals has been reported to cause damage (Azab *et al*., 2008) to various organs of which the liver is the prime target. It has been recognized to cause serious health problems among livestock, where following the early discovery of aflatoxin, Butler (1964) reported that aflatoxin induces hepatic carcinoma at levels as low as 15 ppb.

Aflatoxin has been reported to induce oxidative damage through the generation of free radicals which react with other cellular components in the body resulting pathological changes, degenerative necrotic changes in the kidney and interferes with its functions (Clifford *et al*., 1967). Regarding kidney function, serum creatinine level has been reported to increase significantly in rats that consumed diets with aflatoxin, indicating lower glomerular filtrate rate and a significant reduction of proteins in plasma has been observed too among Newzealand white breed of rabbits (Zahar *et al*., 1996).

Toxins that affect protein components of these organs affect the enzyme production, particularly CP450 which is produced from the liver and kidney cells. In conclusion, the In vivo nutrient digestibilities were significantly affected in the treatments with the toxin, while the kidneys manifested lesions that appeared like congregating inflammatory cells. This may damage the tubules and the functioning of the kidney among the rabbits causing a major health setback to the animal.

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