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Aflatoxin Contamination In Maize Used As Animal Feed For Cattle And Its Potential Mitigation Measures, Current Situation In Kenya

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Abstract: *Mycotoxins are secondary metabolites produced by microfungi that are capable of causing disease and death in humans and other animals. Fungal pathogens of the genera Aspergillus, Fusarium and Penicillium are a major threat to food and feed crops due to production of mycotoxins such as aflatoxins, 4-deoxynivalenol,ergot, patulin, and numerous other toxic secondary metabolites that substantially reduce the value of the crop and subsequent feed meant foranimals.To mitigate against the impacts, it requires immediate necessary control measures. Contamination is generally transferred to animals and human beings when fed with contaminated produce.This review focuses on aflatoxin contamination in maize which is then used in production of animal feed for cattle. The route and the factors increasing the risk of contamination is highlighted and the effects of aflatoxin contamination in cattle as a result of contaminated feed uptake discussed in relation to animal health and production. Aflatoxin contamination has huge impacts as it is a major cause of disease burden and financial losses. Through the evaluation of aflatoxin risk in maize grains used as animal feed, critical contamination points are identified as; field growing, at harvest, storage and transportation stages.*

The review identified three points of mitigating contamination in maize classified as primary, secondary and tertiary activities. The most effective point being deduced as the primary process which minimizes the risk of initial contamination.

Keywords*: aflatoxin, mycotoxin, contamination, mitigating, animal feed*

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I. Introduction

Aflatoxins contaminate many African dietary staples such as maize, groundnuts, rice, and cassava, particularly under certain conditions: dry weather near crop maturity, high moisture during harvest, inadequate drying and storage of crops(Patel *et al., 2015*). In sub-Saharan Africa, climate favours proliferation of fungal species such as *Aspergillus flavus* and *A. parasiticus*. Maize, groundnuts, sorghum, milk and animal feeds are often contaminated with mycotoxins, which are toxic by-products of fungal metabolism (Nleya*et al*.,2018, Lewis*et al*., 2005). Prevalence data from Africa suggests that aflatoxin contamination in maize, groundnuts and sorghum is higher than the European Union aflatoxin standard (4 ppb) and that of USA (20 ppb) in many countries. Importantly to note, is that even aflatoxin exposure at low levels can result in measurable human health impacts due to accumulation, considering that in Kenya the per capita consumption of maize is about 88 kg/capita/year. This means that the Kenyan population may be exposed to regular doses of a wide spectrum of highly toxic, carcinogenic, immunosuppressive, mutagenic, and hepatotoxic mycotoxins through the consumption of maizemeal (Muriuki&Siboe, 1995; Kirimi, *et al*., 2011).

A World Health Organization (WHO) study on 'estimates of the global burden of foodborne diseases' identified global estimates on 31 foodborne hazards which include aflatoxins. Together, the 31 hazards caused approximately 600 million foodborne illnesses and 420,000 deaths in 2010.The global burden of foodborne disease by the hazards was approximately 33 million DALYs. A considerable burden due to aflatoxin was observed in Africa (WHO, 2015). Aflatoxin contamination in grains has been a main challenge in Africa as it poses, not only a challenge to human and animal health, but also it is a food security concern in the countries. The health consequences associated with aflatoxicosis are also far reaching; studies have confirmed aflatoxin contamination to be a known carcinogen (Liu & Wu, 2010,Streit*et al*., 2012, Zain,2011). Additionally, the aflatoxin contamination limits are developed by international trade as a result of strict regulation in high-value markets (Udomkun*et al*., 2017).The European Union for example has set the strictest standards, making it hard to market any product for human consumption with a concentration of AF-B1 and total AFs greater than 2 mg/kg and 4 mg/kg, respectively(EC, 2010).

Kenya has for many years experienced challenges with mycotoxins in a wide range of crops; mainly aflatoxin in maize. This has led to far reaching consequences to human health with reported cases of acute aflatoxicosis. The worst cases of aflatoxicosis wasbetween the months of January–June 2004 in Eastern parts of Kenya which resulted in 331cases being reported and 125 deaths(table no 1). The other parts of the country most affected by aflatoxicosis includes parts of Central Kenya and Coast regions (table no 1). Public health officials sampled maize from the affected area and found concentrations of aflatoxin B1 as high as 4,400 ppb after analysis, which is 220 times greater than the 20 ppb limit for food suggested by Kenyan authorities (Azziz-Baumgartner *et al*., 2005). Animals are also at risk of infection as a result of feed processed from contaminated grain. The effects in animals range from reduced immunity, drop in production to death.Milled maize is a main raw material used in processing of animal feed mainly used in cattle (Kang'ethe&Lang'a, 2009). Aflatoxin contamination in the feed, as a consequence, will lead to exposure in cattle (Shephard, 2009) This in turn increases the risk of aflatoxin M1 and M2 contamination to the milk produced (Streit*et al*., 2012).

Table no 1: Reported cases of aflatoxicosis in Kenyabetween 1981-2010

Year	Affected	Number	Area	Effect	Reference
1981	Humans	12	Machakos	Death	Ngindu, *et al*., 1982
1984/85	Poultry	Large number	Poultry farms	Death	Ngindu*et al*., 1982
1988	Humans	3	Meru North	Death and acute effects	Autrup*et al*., 1987
2001	Humans	3	Meru North	death	Probst, *et. al*., 2007
2001	Human	26	Maua	16 death	Probst *et al*., 2007
2002	Poultry/dogs	Large number	coast	Death	Probst *et al*., 2007
2003	Humans	6	Thika	Death	Lewis *et al*., 2005
2004	Humans	331	Eastern,CentralMakueniKitui	Acute poisoning 125 deaths	Lewis et *al*., 2005
2005	Humans	75	Machakos, Makueni, Kitui	Acute poisoning, 75 cases with 32 deaths	Azziz-Baumgartner *et al*., 2005
2006	Humans	20	Machakos, Makueni, Kitui	Acute poisoning 10 deaths	Muture & Ogana, 2005
2007	Humans	4	Kibwezi, Makueni	2 deaths	Wagacha & Muthomi, 2008
2008	Humans	5	Kibwezi, Kajiado, Mutomo	3 hospitalized, 2 deaths	Muthomi, Njenga, & Gathumbi, 2009
2010	Humans		29 districts Eastern Kenya	Price spiraldown and grain trade breakdown and unconfirmed dog death cases.	Muthomi*et al*., 2010

Source: (Kang'ethe, 2011)

II. Aflatoxin Risk

The Centre for Disease Control (CDC) estimates 4.5 billion people are exposed to aflatoxins worldwide. The risk varies according to the continent and definitely between the different countries; Aflatoxin exposure in Africa ranges from 10 to 180 ng/kg body weight/day, exposures in Europe and North America range from 0 to 4 and from 0.26 to 1, respectively (Liu & Wu, 2010).A study from Kenya shows that populations from all economic strata have high aflatoxin exposure. The level of aflatoxin B1—the most toxic of the aflatoxins—in blood serum was similar across rich and poor, with the highest burden amongst the middle wealth quintile (CDC, 2007)

Climate changes also play a major role and likely to lead to increased occurrence of aflatoxins and other mycotoxins (and possibly their increased co-occurrence) in Kenya and other countries due to variabilities in climate parameters.The tropical and subtropical regionsof the world including sub-Saharan Africa and parts Southern Asia are highly likely to continue experiencing aflatoxin related contamination issues due to high temperatures and humidity conditions being experienced(Nleya*et al*.,2018). This is also true for areas that experience drought as it increases crop susceptibility to aflatoxin contamination (Clarke &Fattori, 2013).

There is a low understanding of the dangers of mycotoxins in food, and that certain practices among farmers may increase the risk for exposure. Gender analysis reveals that groups having knowledge are not always responsible for risk mitigation. In a study conducted in the Kenyan farming regions, sixty seven percent of the urban smallholder dairy farmers had no knowledge that milk could be contaminated with aflatoxin M1 and neither knew how they could mitigate against this exposure. The principal hydroxylated AFB1 metabolite present in most milk of cows fed with a diet contaminated with AFB1is aflatoxin M1(Battacone*et al*.,2003; Applebaum*et al*., 2003). Aflatoxin M1 is usually excreted after 12 hours in milk and urine when animal feed contaminated with aflatoxin is administered to the animals (Jawaid*et al*,2015;Battacone*et al*.,2003). The toxic hydroxylated metabolite M1 is formed as a result of biotransformation of AFB1 and AFB2 by the hepatic microsomal mixed-function oxidase system (Jawaid*et al*., 2015; Ifeoluwa*et al*.,2017). Improper farming practices have led to an increase in risk of contamination like feeding spoilt maize to animals, selling spoilt maize as animal feed, feeding moldy human food to animals, blending of moldy cattle feed with a fresh batch. Very rotten cobs are separated from the good cobs and later shelled separately and the grain used for making animal feeds. The practice is to mix one bag of clean maize with two bags of rotten maize, mill and use these as animal feeds. It was found that this practice of dilution does not drastically reduce the amount of aflatoxin contamination in animal feeds (Kang'ethe&Lang'a, 2009).

The sale of unprocessed milk raises public health concerns due to health risks from pathogens, toxins and drug residues. Although there is legislation in place to control milk safety (Dairy Industry Act, CAP 336; Public Health Act, CAP 242), it is not strictly enforced. Commercial feeds have been found to be contaminated with aflatoxin B1 and milk with aflatoxin M1 (Kang'ethe, *et al*., 2007). The contaminated milk with aflatoxin M1 ultimately causes chronical toxicity in human being as it retains some carcinogenicity from the contaminated feeds (Mohammadi,2011). A study done by Kiama*et al*., 2016 mapped Kenya into risky areas taking into consideration humidity, temperature, rainfall, dairy cattle density, feed resources, farming systems and consumption of maize and milk. The Eastern parts of the country had more cases of historical occurrences of aflatoxin contamination. The Central and Western parts showed increased risk of aflatoxin contamination in crops.

Factors that encourage fungal growth

The growth of fungi is caused by a number of factors which provide the ideal environment that promotes the growth. The conditions must all be present for fungal growth to occur. These conditions are;

- Relative humidity of over 70%
- Temperatures over 30^0C for a period of a few days to a week
- Stress to the affected plant, such as drought, flood, or insect infestation
- High moisture content of crop (20% or higher)

Contamination pathway

In the Kenya dairy value chain, milk production is mainly from dairy cattle which are normally fed on natural forage, cultivated fodder and crop by-products such as maize stalks and stover. Commercially available supplements include dairy meal, maize germ, maize bran, cottonseed cake, wheat pollard and wheat bran. Urban dairy farmers in Kenya have been shown to spend nine times more money to purchase commercial feeds than their rural counterparts (Staal,*et al*., 2003) and are at a higher risk of feeding AFB1-contaminated animal feeds. Maize crops undergo avalue chain from the time they are in the field to the point of maturity, harvest, storage, transportation and subsequent distribution to market places or to animal feed companies (Kiama*et al*., 2016). Others are used for own consumption and for animals. Throughout this chain, there is a risk of occurrence of growth of fungi and contamination with aflatoxin. Contamination will be encouraged by favourable conditions present or occurring in sequence along the value chain. These conditions are;

1. Crops in the field- biological factors which include a susceptible crop, presence of a compatible toxigenic species.
2. Environment- temperature, moisture, mechanical injury, insect/bird damage and presence of fungus.
3. Harvest- crop maturity, temperature, moisture and damage e.g. during shelling.
4. Storage- attack by insects, moisture and temperature.
5. Transport and distribution- handling practices, damage, mixture with already contaminated maize.

These factors increase the risk of contamination of the final product that goes to the final consumer; in this case animal feed intended for cattle. A study in the Rift Valley part of Kenyafound that maize is dried along the road sides or in open fields where soil is easily brown onto the drying maize on canvas thus increasing the risk of contamination by spores present in the soil.The small traders buy maize directly from smallholder farmers and assemble in bulk to deliver to small market retail traders, large trading companies or maize millers. The small traders often don't have a very good understanding on the implications of aflatoxin testing because of the volumes they handle. Also, the clients may not require the assurance that the maize meets the standards on aflatoxin. Most private sector farmers rarely test their maize for aflatoxin contamination unless it is demanded by the buyers. Only grading checks are carried out by a majority of maize tradersbut no confirmatory tests for aflatoxin (Kang'ethe, 2011). This creates a huge of products which are highly contaminated from the raw materials as confirmed in a study by Okoth& Kola,(2012) which found maize products in the Kenyan markets to be highly contaminated with aflatoxins.

The compounded contamination pathways from the field, harvest, storage and distribution to markets leads to an increased level of contamination to the animal feed processed from the maize. In the 2004-2006 outbreak, poor post-harvest handling especially storage at household level was blamed for the outbreak.

Maize contamination in the value chain will lead to consequences which can be grouped into health hazards and negative economic impacts i.e.low productivity, loss of market, loss of produce and decreased price for produce.

In Kenya, maize production is produced for three main reasons; market, own consumption and livestock feed. Contamination at source will impact negatively on the 3 uses as illustrated in table no 2.

Table no 2: Health and economic effects of maize contamination

Hazard	Maize Production		
	Market	Own consumption	Livestock feed
Contamination	**Market loss** • Supply shortage • Discarded grain • Litigation	**Human health** • Disease burden • Reduced productivity	**Market loss** • Reduced milk productivity • Livestock disease burden • Reduced prices of products e.g. cheese • Discarded products

Contamination of animal feed will to a large extent affect the health of cattle consuming such feed. This will impact negatively to the health and production of the animal. The consequences include;

- decreased feed intake and efficiency
- lower milk production
- gastroenteritis which leads to complications such as intestinal hemorrhages, impaired rumen function, ketosis and diarrhea
- impaired reproduction coupled by irregular heats, low conception rates, ovarian cysts and embryonic loss
- production – milk contamination, low production and mastitis, andlaminitis which causes low mobility in grazing cattle

III. Mitigation Of Aflatoxin Contamination

These are methods which can be used to avoid and/or control aflatoxin contamination in maize. The occurrence of aflatoxin can be at either the pre-harvest stage or during harvesting stage (Songsermsakul,2015). It being a stable metabolite,to remove it from an already contaminated feed is hard, mitigation of its occurrence seems to be the best control option(Galla*et al*.,2015). The mitigation and /or control methods can be grouped majorly asprimary, secondary and tertiary.

Primary: This is the most important and effective control measure since it is initiated before fungal growth. This involves observance of good farming practices e.g. Good Agricultural Practice (GAP) andHazard Analysis Critical Control Point (HACCP). It focuses on keeping the conditions unfavorable for growth of toxin producing molds. Some of the farming practices are crop rotation and since maize is susceptible to *Fusarium* infection, it should be avoided as pre-crop to other *Fusarium* sensitive crops (Jouany, 2007). The primary mitigation method might involve the use of biological organisms as a control measure towards aflatoxin. This involves the use of non-aflatoxin forming strains of A. *flavus*, which competes with both toxigenic and other atoxigenic strains in the soil, for the infection sites and essential nutrients needed for growth (Cotty,2006). The inoculation of the non-toxic agents in the soil to control fungal growth has a carry-over effects which prevents the harvested products from contamination during storage (Dorner and Cole,2002). Aflasafe™ is an example of biocontrol agents developed and proven to be successful in the control of aflatoxin in maize and groundnuts in Kenya and otherSub-Saharan African countries (Grace *et al*.,2015; Bandyopadhyay and Cotty, 2013).

Secondary: This takes place if invasion of some fungi begins in commodities at an early phase. The aim is to eliminate toxigenic-fungi and stop its growth. It involves re-drying products, sorting to remove contaminated grains, inactivation (may include thermal, chemical or use of toxin binders), exclusion of favorable growth conditions e.g. ventilation in storage granaries, control of insects and rodents(Fandohan*et al*.,2005).Bentonite clays, suchas montmorillonite,for example can be used to mitigate the adverseeffects of aflatoxins in contaminated diets and diminishaflatoxin carry-over to milk of lactating animals through adsorption (Phillips *et al.*, 2006).

Sorting by rejection of the damaged samples among cereals reduce aflatoxin contamination. Sorting in groundnuts and pistachio nuts by floating and density separation and fluorescence sorting has reported to have reduced aflatoxin contamination by 95% (Shakerardekani*et al*.,2012;Phillips *et al*.,1994;Tyson and Clark,1974).Proper use of the storage material can also prevent fungal growth. During storage and distribution, maize and other food products might be contaminated by the aflatoxin if conditions which favor fungal growth such as temperature and humidity are present (Giorni*et al*.,2008). To avoid possible contaminations of aflatoxin, smallholder farmers need to change from the traditional storage methods to modern methods which will ensure safe storage of their produce. They are however faced with high costs and difficulties with accessibility making adoption of these practices limited (Hell and Mutegi,2011).

Tertiary: This is done on heavily infested products by toxic fungi. The measures are taken to prevent the transfer of fungi and their health hazardous toxins. Common practices are alkalinization, complete destruction of the contaminated grain, detoxification/destruction of toxins to minimum levels (this can be physical, chemical or biological).Treatment of contaminated maize with radiations such as gamma rays can be an effective method to destroy the fungi responsible for aflatoxin (Jalili, 2010). The high energy gamma radiations destroy the damage to DNA in microbial cells (Markov *et al*., 2015). Additionally, they produce free radicals and ions that attack the DNA of microorganisms through the interactions with water molecules present in the substrates (Da Silva Aquino, 2012). Other tertiary non thermal methods used for the treatment of aflatoxin in maize include use of UV-VIS radiation and pulsed light.

Use of chemicals to control the infested cereals has been successful despite being faced with challenges of resistance (Hontanaya*et al*.,2015).Studies involving the use of citric acid and sodium hydrosulphite on sorghum and red pepper respectively resulted in a greater percentage reduction of AF-B1, AF-B2, AF-G1, and AF-G2 (Mendez-Albores*et al*., 2009; Jalili and Jinap,2012). Combination of 2, 6-di (t-butyl)-p-cresol (BHT) and the entomopathogenic fungus *Purpureocilliumlilacinum* was significantly found to reduce the accumulation of AF-B1 in stored maize (Barra *et al*.,2015), hence acting as a potential and successful strategy to control aflatoxin contamination.

If farmers have to dispose their contaminated produce, safe disposal methods which are economically sensitive to farmers should be available e.g. using the produce as fuels or blending to animal feeds as long as the allowable limits are achieved. This will help in reducing the losses incurred by farmers. It will also help bridge the compliance gap as naturally no one would want to knowingly sell contaminated produce in the market.

Other mitigation approaches

Information dispatch and training of farmers through open field days should be done regularly and intensified during the harvest period. This will help farmers implement primary control measures and will also be in a position to detect early growth of molds in their maize (Strosnider*et al*.,2006). It is necessary to have enhanced laboratory capacity and availability of rapid test kits, trained users, documentation of results, and withdrawal of contaminated products to enable greater separation of contaminated crops in markets. Enhanced laboratory capacity will promote carrying out more regular testing of aflatoxin levels in major foods, and establishing reference laboratories for mycotoxin studies. In this regard, rapid test kits can be given to extension officers who can use them in maize distribution points or on mold infested maize at farm level.

It is widely recognized, however, that reliance on testing is an inefficient and ineffective approach to the control of food contaminants. In particular, aflatoxin contamination is notoriously heterogeneous, which increases the difficulty of estimating true contamination levels of affected lots. Adopting good practices at all stages of the food chain to minimize infection by toxigenic molds and the accumulation of mycotoxin contamination is the best way to reduce levels of these fungal toxins in the food supply.

Farmers' trainings on proper storage of harvested maize will be key in reducing the levels of aflatoxin in maize as most cases of contamination are reported during the storage period (Gehesquière*et al*.,2016).The primitive methods used in shelling the maize, increases their exposure to fungal infection (Mubatanhema*et al*.,1999). Farmers' awareness on proper maize processing practices and storage will therefore act as a way of mitigating aflatoxin contamination in maize.

Promotion of animal health through use of aflatoxin-safe feed or chemical toxin binders and anti-caking agent should be prioritized. To prevent the harmful effects of aflatoxins in animals, chemical compounds and polymers known as 'binding agents' can be added to animal feed for pennies on the metric ton of animal feed.

Overall, it is important to put in place an effective regulatory regime that ensures compliance by all stakeholders. This involves a food safety control system upgrade which ensures an all-inclusive legislation which includes country-specific standards that account for consumption patterns building on Codex Alimentarius and consistent with the World Trade Organization Sanitary and Phytosanitary Agreement (PACA,2013).

IV. Recommendations

Despite the aflatoxin problem being recognized for many years, including the loss of lives, the Kenyan government has not put in place any official aflatoxin surveillance and monitoring programmes.Therefore,it is important to have a proper preventative programme to address the aflatoxin problem which would lead to a reduction in aflatoxin contamination in maize at the beginning of production and not focusing on treatment at the end. Over the years, the government has been involved in various interventions activities including farmers' education at the rural level. Future interventions should however be targeted at proper timing throughout the whole maize growing cycle.

It has been noted that if there is general awareness of aflatoxin in a country and there are supporting regulations and institutions, then the human health impact of aflatoxin contamination will be low but market impact will be high. On the other hand, if awareness is low and there are inadequate regulations to control it, aflatoxin-contaminated grain will trade freely, in which case the health impacts will be high. The majority of maize production in Africa is used for a producer's own consumption, implying that the human health impact will be the greatest if there is lack of awareness about aflatoxin and this risk will with no doubt directly translate to contamination of animal feed produced from contaminated maize(PACA, 2013). Hence, awareness on aflatoxin prevalence and contamination should be continuously promoted across the country with special focus on susceptible regions.

Governments are key players who should provide the financial resources necessary to run monitoring and surveillance programs. Since susceptible regions in Kenya have already been identified, conducting population monitoring and mapping of the exposure to aflatoxins should be the next step once a surveillance program is in place and should be enhanced during susceptible periods of harvest, storage and distribution.

Effective national programs for reducing aflatoxin contamination require an awareness of the international standards on allowable levels of aflatoxin in grains and how they are developed, an adequate regulatory framework that enables implementation and enforcement of relevant standards. For example, there is a disjoint between the FAO/WHO limits of aflatoxin in maize and milk and theKenya Bureau of Standards (KEBS). These should be harmonized to help Kenyan produce (including milk products) access international markets.

The necessary support to facilitate uptake of the good practices by value chain operators is also required. Practical areas of intervention by the government include establishing mobile maize drying units or construct driers in specific areas where farmers can access and have their maize dried before storage. At the same time, provide/subsidize simple testing kits like the digital moisture analyzers which can be placed at convenient collection points. Trained farmers or extension officers in collaboration with the National Produce and Cereals Board (NCPB), which has ready access to farmers in maize growing regions,can make use of the analyzers before maize is stored or distributed.

V. Conclusion

A One Health approach is required to fully address the aflatoxin menace which continuously affects many Kenyans. Various stakeholders including the government through its agencies and farmers themselves should interact widely in coming up with practical and long lasting solutions. These interventions should be timely and economically viable because aflatoxin contamination is a food security problem as the hunger stricken areas would rather consume or sell the contaminated maize than destroy it.

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