Assessment of Aflatoxin in Dairy Feeds, Milk, Blood and Evaluation of Mitigation Measures in North of Rift Valley Counties, Kenya

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Abstract

Aflatoxin is a group of mycotoxins produced by fungi that grow on food and feed materials. They are poisonous to animals and humans if consumption exceeds permissible levels. Their detrimental effects include drop in general animal productivity, increased disease incidences, chronic damage to vital organs and decreased reproductive performance. A survey was done in the North Rift region to investigate the prevalence of aflatoxin in dairy feeds. Feed samples from 16 farmers in three counties were collected by purposive sampling. The samples from the three counties were analyzed for aflatoxin B_1. A four by four Latin Square experiment to assess the efficacy of calcium hydroxide, ammonium carbonate and brewer's yeast to reduce aflatoxicosis in dairy cows was done. Samples of milk, blood and faecal material were collected at the end of each 7-day period. Feed and faecal samples were analyzed for aflatoxin B_1 while milk and blood samples were analyzed for aflatoxin M_1 using ELISA procedure. Statistical analysis of data on survey samples was done using SPSS for descriptive statistics while analysis of variance of experimental data was by SAS. The study revealed that all second grade maize samples obtained from the region was contaminated with aflatoxin B_1, the highest concentration of 15.5 ppb being recorded in Cherangani while lowest concentration of 0.01 ppb was in Nandi County. The overall means for aflatoxin concentration was 1.210 ppb.The effects of calcium hydroxide, ammonium carbonate or brewer's yeast milk production, Aflatoxin M_1 in milk and in blood were not significant ($P>0.05$), but significantly altered the amount of aflatoxin B_1 in faeces ($p< 0.05$). The aflatoxin M1 content in milk and blood was 0.196 and 0.018 ppb respectively. The percentage of feed aflatoxin in milk and blood was 0.997 % and 0.078 % respectively. Treatment of alkaline compounds and Brewer's yeast as used in this study did not reduce aflatoxin M_1 in milk and blood but, had a significant effect on aflatoxin B_1 in faeces.

Keywords: *Aflatoxins, dairy feeds, dairy cows, blood, milk*

Introduction

Background

Mycotoxins are chemical compounds produced by some fungi species as a form of defense. There are over 400 types of mycotoxin known in the world. The type of mycotoxin produced depends on the specie of fungi present (Binder *et al.,* 2007). A fungus produces mycotoxin when they invade the crop in the field or under storage. *Fusarium sp* and *Aspergillus sp* are the major mycotoxin producing fungi. The mycotoxin synthesized finds their way into feeds and food chain (Neyole *et al.,* 2008).

Animals that consume this mycotoxin beyond maximum recommended levels are at risk of experiencing toxic effects which include mutagenic, teratogenic and carcinogenic effects. Economic losses incurred are decline in feed intake, increased disease incidences, chronic damage of vital organs and decreased reproductive performance. In animal production, deoxynivalenol, Zeraelenone, Ochratoxin, Fumonisins, Aflatoxin, Vomitoxin and Ergot alkaloids are hazardous (Binder *et al.,* 2007).

Domestic animals that consume mycotoxins pass the metabolites residues in animal products such as milk, meat and eggs (Neyole *et al.,* 2008). During metabolism, several products with varied toxicity are formed. Bii (2013) asserts that the levels of mycotoxin along the Kenyan food web is unacceptable and there is no way we can attain food security targets without addressing food safety.

Lukuyu *et al.,* (2009) revealed that farmers and feed mills use oil-seed byproducts such as cakes and second grade maize to feed their animals. These are sometimes contaminated with Afla B_1 (Neyole *et al..,* 2008; Bii, 2013). The mycotoxin concentration in the ingredients can easily lead to extremely high levels in the final feed. The mycotoxins lowers feed quality, feed conversion efficiency and also lowers milk quality and quantity (Lukuyu *et al.,* 2009). Bii (2013) indicates that mycotoxins are present in beer, fermented milk (mursik) and traditional beer (busaa). Humans are indirectly exposed to mycotoxin

metabolites in grains and their products as well as milk and other animal food products. Aflatoxin M_1 (Afla M_1) in milk is a metabolite of Aflatoxin B_1 (Afla B_1) in feed (Lukuyu *et al.*, 2009).

Mycotoxin binders to minimize the effects of aflatoxin have been proposed for use in contaminated feeds. Physical binders such as clays and activated carbon (Diaz *et al*., 2004), biological control such as brewer's yeast and enzymes and the use of alkaline compounds (Elias *et al*., 2002) have been proposed but with inconsistent efficacy.

This study, sought to investigate the level of aflatoxin B_1 in second grade maize grain used in dairy feeds in the North Rift, determine the level of Aflatoxin B_1 in a formulated dairy feed and determine the effects of including ammonium carbonate, brewer's yeast or calcium hydroxide in aflatoxin B_1 contaminated feed on milk production and the levels of Aflatoxin B_1 in faeces, milk and blood of dairy cows.

Materials and Methods

Study Site and Field Survey

The survey study was done in Nandi, Uasin Gishu and Trans Nzoia Counties in North Rift region of Kenya. Either most of the land in these counties lies in UM or LH zones (Rees *et al.*, 1997). The majority of the small scale farmers do not formulate their own dairy meal but use second grade maize as supplement. 48 samples of second grade maize were collected from 48 households in TransNzoia, Uasin Gishu and Nandi Counties. Each county was stratified into four stratus based on the political administration. Within each stratum, at least four samples were collected through purposive sampling from small, medium and large scale farmers. Sampling was done with the help of District Agricultural and Livestock extension officers. The samples were ground such that 50 % could pass through a 1 mm mesh screen. The machine was cleaned before and after each sample. The ground samples were packed in plastic bags and refrigerated till laboratory analysis.

Experiment with dairy cows

TMR Formulation

All the required ingredients were assembled. A sample of 100 g of each ingredient was taken and analyzed for the level of Afla B_1. The ingredients were emptied into a 2.5 tonne Feed Mixer in the following ratio as indicated in Table 1. The composition ranged from 1.25 (vegetable oil) to 27% (Rhodes grass hay).

Table 1. The composition of the Total Mixed Ratio (TMR) in percentage

Ingredients	**Composition (%)**
Hay(Rhodes Grass)	27.00
Sunflower Cake	22.53
Second grade maize Grain	19.50
Maize Germ Meal	15.20
Wheat Pollards	5.80
Mineral(Mac lick Super)	4.00
Fish Meal(Omena)	3.82
Urea	0.95
Premix	0.80
Vegetable Oil	1.20
Total	**100**

Feeding Experiment and Data Collection. Selected cows moved to experimental units and preconditioned for seven days. The experiment design was 4 by 4 Latin square indicated in Table 2.

Table 2. Feeding experiment design

	COWS			
	Cow 1	Cow 2	Cow 3	Cow 4
Period 1 (Day8-14)	Diet A	Diet B	Diet C	Diet D
Period 2 (Day15-21)	Diet B	Diet C	Diet D	Diet A
Period 3 (Day 22-28)	Diet D	Diet A	Diet B	Diet C
Period 4 (Day 29-36)	Diet C	Diet D	Diet A	Diet B

Milk Samples Collection. On day seven of each period, 200 ml of milk was sampled from each cow. This was obtained by taking the first drops from each quarter of the udder and mixing them. A representative sample was obtained by further sub sampling. The subsample was refrigerated for 2 hours and the centrifuged at 2000 g for 5 minutes to induce separation of upper fatty layer. The upper fatty layer

was removed by aspiration and the lower plasma used in the assay. Quantification of Aflatoxin M_1 was done as outlined in Helica Aflatoxin M1 CAT.NO.961AFLMO1M.

Blood Samples. On the seventh day, seven of each period 100 ml of blood was sampled from each cow. This was taken from the jugular by vein puncture. The blood sample was centrifuged at 2000 g for 5 minutes to induce separation of blood plasma (serum) from other blood components. The blood serum was obtained by aspiration and used in the assay. The assay for quantification of Afla M_1 was as outlined in Helica Aflatoxin M1 CAT.NO.961AFLMO1M.

Faecal Samples. Faecal samples were obtained from the rectum of each cow on mornings of days 7, 15, 22, 29 and day 36. The faecal samples were oven dried for twenty-four hours and a sub samples taken for Afla B1 extraction. The extraction solution was prepared by adding 70 ml methanol to 30 ml of distilled water. The representative sample was ground to a particle size of instant coffee (50% passes through a 20-mesh screen), then 20 g of the ground sample was added to 100 ml of the extraction solvent. The ratio of the sample to extraction solvent is 1:5 (w/v). The mixture was sealed in a beaker for 2 minutes and allowed to settle, then 10 ml of filtrate extract was collected through Whatman # 1 paper. The quantification for aflatoxins B_1 using Elisa Kit was done as outlined in Helica TOTAL AFLATOXIN B_I ASSAY (CAT.NO.9414AFLO1M-96).

Statistical data analysis. Results of aflatoxin concentration in field samples were statistically analyzed for descriptive statistics at $P \leq 0.05$. The feeding experimental design was a four by four Latin square. The means for aflatoxins in faeces, milk and blood were analyzed for variance using SAS edition 9.1.3, and means were separated using Fisher's Least Significant Difference (LSD) at $P \leq 0.05$.

Results

The Feed survey samples Results

The means of Afla B1 in the samples collected was 1.738±0.966 ppb, 1.06±0.177 ppb and 0.834±0.213 ppb for TransNzoia, Uasin Gishu and Nandi counties respectively as indicated in Table 3. The highest levels were registered in Trans Nzoia (AEZ (UM) and the least was in Nandi county (AEZ LH). There was great variability in Trans Nzoia samples as seen from SD and CV compared to other counties.

Table 3. Means of Afla B_1 levels in second grade maize samples

County	N	Mean (ppb)	STD	CV	Range	
					Min	Max
Trans Nzoia	16	1.738±0.966	3.73	215.1	0.01	15.5
Uasin Gishu	16	1.060±0.213	0.684	64.60	0.01	2.61
Nandi	16	0.833±0.177	0.823	98.75	0.01	4.30

Aflatoxin B_1 Concentrations in a TMR

A TMR was formulated for the purpose of conducting an experiment. The TMR met the minimum nutrients requirement for a 400 Kg cow (NRC, 2001). The TMR was used as a basal diet in feeding four lactating Holstein Friesian cows. The TMR had aflatoxin level of 20.5 ppb.

Daily milk production by cows subjected to the four treatments

The mean milk production per day was as indicated in Table 4 below. This difference was not statistically significant at $P \leq 0.05$.

Table 4. Average milk yield per day during the experiment

		Diet	Compositio Average
A	TMR + 2 % Brewer's yeast	n	
B	TMR + 2 % Brewer's yeast + 1 % CaOH	(Kg)	
C	TMR + 2 % Brewer's Yeast + 2% NH_3CO3	13.34 a	
D	TMR	13.06 a	
*Sem = ±0.68, means with the same letter are not significantly different		12.97 a	
Level of Afla B_1 in Faecal Samples		12.20 a	

Efficacy of the Diet was tested by analyzing the level of Afla B_1 in faeces. The faecal sample from animals fed different Diet was significantly different at $p \leq 0.05$. The highest level was found in Diet D at 4.750±0.558 ppb while Diet B and C had the same level at 2.000±0.558 ppb. Diet D was a control while Diet A was treated with brewer's yeast. Diet B and Diet C had CaOH and NH_3CO_3 added to their diet respectively.

Level of Afla B_1 in Blood Samples

Despite exposing the four diets to different condition there was no significant difference in the level of Afla M_1 in blood samples as indicate in table 8 below. The mean level of Afla M_1 in blood serum was 0.016±0.004 ppb for Diet A, B, C while Diet A had 0.023±0.004 ppb.

Level of Afla B_1 in Milk Samples

The level of Afla M_1 in milk was statistically insignificant as indicated in Table 5.

Table 5. Means of aflatoxins in basal diet, faeces, milk and blood serum obtained from cows during the experiment

	Aflatoxin B_1 in feed (ppb)	Aflatoxin B_1 in feaces (ppb)	Aflatoxin M_1 in milk (ppb)	Aflatoxin M_1 serum (ppb)
Diet A	20.5	3.125 ab	0.199a	0.016a
Diet B	20.5	2.000 b	0.199a	0.016a
Diet C	20.5	2.000 b	0.198a	0.016a
Diet D	20.5	4.750 a	0.188a	0.023a
SEM		±0.558	±0.007	±0.004

*Means with the same letters are not significantly different

Relative Percentage

Data from samples collecting during the experiment was used to trace the partitioning of Afla B_1 ingested (100 %) as shown in Table 6. Table 6 indicates that the control diet had the highest level of excreated Afla B_1 (23.17 %) followed by Diet A (15.24 %) but Diet B and C were least each at 9.76 %. In blood sample, the level in Afla M_1 metabolite in blood serum was relatively stable at 0.078 % with the exception of the control (0.112 %); this was statistically insignificantly different $P \leq 0.05$.

Table 6. The relative percentage of aflatoxin that appears in faecal, milk and blood serum

Diets	Aflatoxin B_1 in diets (%)	Afla B_1 in faecal (%)	Aflatoxin M_1 in milk (%)	Aflatoxin M_1 serum (%)
A	100	15.24	0.970	0.078
B	100	9.76	0.970	0.078
C	100	9.76	0.965	0.078
D	100	23.17	0.916	0.112

Discussion

The Prevalence of Afla B_1 in animal feeds in North Rift region

All the samples of second grade maize collected from farmers were contaminated with Aflatoxin B_1 (Afla B_1). This confirms the presence of aflatoxin in this region. Neyole *et al*., (2008) found out that up to 30 % of grain loss was due to moulding. Similarly, Bii (2013) showed that 100 % of maize and oil cakes used for feeding livestock in the neighbouring South Rift was contaminated with Afla B_1.

The highest level was reported in Trans Nzoia County at 15.5 ppb which above the maximum set limit of 10 ppb. This probably due to difference in agro- ecological zones and other economic activities besides dairy farming. Nandi County majorly practice tea farming while TransNzoia and Uasin Gishu County were cereal oriented hence the population of *Aspergillus spp.* fungi were lower in Nandi County. Kang'ethe *et al*., (2009) revealed 67 % of dairy concentrates had Afla B_1 levels above 20 ppb. This study focused on second grade maize where all the samples were positive of Afla B_1. Only 6.25 % of the samples exceeded the maximum limit set by European Union at 3 ppb for human consumption. According to Bii (2013), oil seedcake were contaminated and thus using second grade maize and oil seed cakes would easily result in a concentrate with a concentration above maximum set by Kenya Bureau of Standards of 20 ppb. The finding of this research are consistent with International Food Policy Research Institute (IFPRI) (2011) which revealed that maize from Western Kenya had Afla B_1 ranging from 0 ppb to 1.4 ppb.

The Effect of Diet on Afla B_1 Excreation in faeces

Statistical analysis showed a significant difference in the level of Aflatoxin B_1 excreated in faeces ($P \leq 0.05$). Diet D gave the highest ($p < 0.05$) level of Aflatoxin B_1 in faeces. This level was not higher than that of Diet A ($p > 0.05$). The level of Aflatoxin B_1 in faeces from cows fed on diet A and D was significantly higher ($p < 0.05$) than that from Diets B and C. There was no significance differenced in the levels of Aflatoxin B_1 in the basal diet. The insignificant difference between faecal samples from control Diet and other Diets is probably due to addition of brewer's yeast. The further difference between Diets A vs B&C is associated with further exposure to 2 % CaOH and 1 % NH_3CO_3 respectively for the later respectively.

This study found out that 23.17 % of the oral dose was detected in faeces. Helferich (1986) found out that about 50 % of the oral dose was detected in faeces of dairy goats. Polan *et al.,* (1974) fed lactating cows on radio labeled ^{3} (H) Afla B_1 monitored and recovered 5 % in faces. The figures of for the level in the faeces are approximately close to the findings by researchers who did similar studies. CaOH and NH_3CO_3 which were the treatment of diets Diet B and C respectively could have converted Afla B_1 into other products that the study never investigated. However, the level added could not cause a significant difference compared with Diet A. In vitro studies had shown alkaline could reduce aflatoxin B_1 levels by 42-50 % (Var *et al*., 2008), also lack of consistency could be due to variation in the *in vivo* and *in vitro* environment.

The Effect of Diets on Aflatoxin M_1 (Afla M_1) in Blood

There was no significant difference in the level of Afla M_1 in blood samples extracted from the cows during the experiment $P< 0.05$. The mean was 0.0175 ppb and this represented a relative percentage of 0.078 %. Helferich (1986) recovered 1 % of Afla M_1 in milk 120 hours after administration.

The level in blood was lower than that in milk, however during lactogenesis, the process of milk synthesis and extraction may concentrate Afla M_1 in milk from the blood against a concentration gradient.

The Effect of Diet of Aflatoxin M_1 (Afla M_1) in Milk

The Diets had no significant effect on the level of Afla M_1 excreated in milk. At a mean of 0.196, this was about 0.970% of what was in the diet. The numerical difference for the control Diet is statistically insignificant. Helferich (1986) found out that 0.7 to 1.4% of Afla B_1 in basal diet was excreated in milk. Recent study by scientist in Israel indicate that 1-2% Afla M_1 for cows producing below 30 kg/day of milk and 4-6% Afla M_1 for those producing above 30 Kgs/day of milk (Malka *et al*., 2013). The average Afla M_1 concentration in milk was 0.97 % an approximately 1 %. The carryover effect of aflatoxins in milk is influenced by difference in species (Battacone *et al.,* 2003), individual animal variability and integrity of alveolar cell membrane of mammary gland, milk yield (Malka *et al*., 2013), stage of lactation and mammary gland infection (Veldman, 1992).

Lacks of significant difference between the cows shows animals that yield the same amount of milk per day have similar carryover effect. The Diet of feeds with brewer's yeast, CaOH and NH_3CO_3 at the levels used in this study has no effect of absorption, metabolism of Afla B_1 and excreation of Afla M_1 in milk. However this is restricted to the levels used, this study used 2 % for CaOH and brewer's yeast while 1 % NH_3CO_3 was at 1 %. This contrast with the previous studies carried out by various scholars. Elias-Orozco *et al.,* (2002) indicates that alkaline compounds like CaOH and sodium hydroxide could reduce Afla B_1 in vitro by 41±3.7 %- 55.3±2.0 % . The research chose CaOH because of the advantage of calcium to dairy animals. The lack of significance difference is probable due to slight acidic environment that exists in the rumen of the cows. Alkaline compounds can open the ring of Afla B_1 and transformed it to a compound called beta-keto acid which is water soluble hence can easily be washed away by water (Parker & Melnick,1966;Tabata *et al.*,1994).

Murphy *et al*., (2006) reported that brewer's yeast can reduce aflatoxin in vitro by up to 90 % in 90 minutes, this study included brewer's yeast at 2 % but this had no significant different with regard to aflatoxins in faeces, milk and blood serum compared to the control and other Diet. This consistency with Hashmi *et al*., (2006) showed that there is no significant difference on the level of aflatoxin in the liver of a broiler at 1 % inclusion of brewer's yeast for diets containing 100 ppb, 200 ppb and 300 ppb.

Conclusion and Recommendations

This study has revealed that all second grade maize used by farmers to supplement dairy production in North Rift is contaminated with aflatoxin. Using second grade maize alone to supplement dairy production cannot cause acute toxicosis in cows because the levels are below maximum limits set by the EU. Using CaOH, NH_3CO_3 and brewer's yeast at levels used in this study are not effective in reducing aflatoxins in dairy products. The level of carryover effects of aflatoxin in milk is approximately 1% of that in the basal diet.

It is therefore recommended that:

1. Farmers should be discouraged from using large quantities of second grade maize in feeding their dairy cows: this will enhance the quality and safety of milk.
2. There is need to assess the level of other mycotoxin in dairy feeds especially in North Rift since mycotoxins act synergistically.
3. There is need to study the effective level of inclusion of alkaline compounds and brewer's yeast to feeds before embracing this technology.

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References

Battacone, G., Nudda A., Cannas A., Borlino, A.C., Bomboi., G & Pulina G (2003). Excretion of aflatoxin M_1 in milk of dairy ewes treated with different doses of Afla B_1. *Journal of Dairy Science 86*, 2667–2675.

Bii,C.(2013, February 14). Poison on the table: We are eating ourselves to death,study reveals. *The Standard Extra*, p 1-4. Nairobi: Standard Group.

Binder, E.M., Chin, L .M. Hand, J. Richard, J. (2007). Fusarium and their mycotoxins: Mycology, occurrence, toxicity, control and economic impact. *Journal of Animal Science and Technology, 137*(3-4), 265-282.

Diaz, D.E., Hagler, W.M., Blackwelder, J.T. Eve, J.A., Hopkins, B.A., Anderson, K.L., Jones, F.T & Whitlow, L.W. (2004). Aflatoxin binders II: reduction of aflatoxin M_1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*, 157, 233-241.

Elias-Orozco, R.., Castellanos-Nava, A., Gayta´ n-Martı´nez M, Figueroa-Ca´ rdenas JD, Loarca-Pin˜ a., G. (2002). Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content. Food Additive Contaminants. 19:878–885.European Commission. 2006. Commission Regulation (EC) No. 401/2006. *Journal of European Commission, 70*, 20–21.

Helferich,W.G., Baldwin, R. & Hsieh, D.P.H.(1986). Aflatoxin B_1 metabolism in lactating goats and rats. *Journal of Animal Science 62*, 697-705

IFPRI (2011). *New study documents Spread of Aflatoxins in Kenya.* Nairobi: Author. Link http//www.ifpri.org

Kang'ethe, E.K., & Lang'a, K.A. (2009). Aflatoxin B_1 and M_1 Contamination of animal feeds and milk from urban centres in Kenya. *African Health Science 9*(4), 218-226

Lukuyu, B. A., Kitalyi, A., Franzel, S. Duncan, A. & Baltenweck, I. (2009). Constraints and options to Enhancing Production of high quality feed in Dairy Production in Kenya, Uganda and Rwanda. *ICRAF Working Paper no. 95*. Nairobi: World Agroforestry Centre.

Malka, B., Shmulik, F., Joshua M., Ran, S .,Olga, C., Jakob, A., Shimshoni, Stefan, S., Rina, A., Sima, A & Alan, S.(2013 **)**. *Carry-Over of Aflatoxin B1 to Aflatoxin M_1 in High Yielding Israeli Cows in Mid- and Late-Lactation. Toxin* 2013 5, pp 173-183. Accessed on 15 May, 2013 link http/www.mdpi.com/journal/toxins

Murphy, P.A., Hendrich, S., Landgren, C., & Bryant, C. M. (eds.) (2006). Food mycotoxins: An update. *Journal of Food Science, 71*, R51-R65.

National Research Council, (2001). *Nutrient Requirements of Dairy Cattle* (7^{th} rev. ed.). Washington, DC, USA: National Academy Press

Neyole, E.M. & Maiyo, A. (2008).Aflatoxin risks in maize production and its impact on food security in Rift valley Kenya. *International Journal for Disaster Management & Risks reduction, 1* (2), 13-19.

Rees, N. & Wandera (Eds.). (1997). *A review of agricultural practices and constraints in north of Rift Valley Province in Kenya.* Kitale: Kenya Agricultural Research Institute.

Statistical Analysis System Institute.(SAS)/STAT user's guide: statistics, version 9.1. 3. Statistical Analysis Systems Institute.

Tabata, S., Kamimura, H., Ibe, A., Hashimoto, H &Tamura, Y. (1994). Degradation of aflatoxins by food additives. *Journal of Food Protection, 57*, 42–47.

Var, I., Kabak B., & Erginkaya Z. (2008). Reduction in Ochratoxin levels in white wine, following treatment with activated carbon and sodium bentonite. *Food Contamination, 19*,592–598.

Veldman, A., Meijs, J.A.C., Borggreve, G.J & Heeres van der Tol J, J. (1992). Carry-over of aflatoxin from cows' food to milk. *Animal Production, 55*, 163–168.