

The Contamination of Kenyan Lager Beers with *Fusarium* Mycotoxins

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ABSTRACT

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Seventy five samples of two popular lager beers, namely Pilsner and Tusker were randomly collected from the city of Nairobi and the surrounding satellite towns in Kenya. The samples were analyzed for the presence of 4 mycotoxins, namely, deoxynivalenol (DON), fumonisin B₁ (FB₁), zearalenone (ZEA), and aflatoxin B₁ (AFB₁), by the competitive enzyme linked immunosorbent assay (ELISA) technique. The incidences of DON and ZEA were 100% in both brands, while for FB₁ the incidence was 72%, with incidences in Tusker (76.9%) being significantly higher than in Pilsner (66.7%) ($p = 0.00$). The mean values for contamination were 3.29 and 3.57 ng/mL for DON, 0.28 and 0.32 ng/mL for FB₁ and 7.84 and 8.50 pg/ml for ZEA in Tusker and Pilsner brands respectively. A positive occurrence association was found between DON and FB₁ and DON and ZEA, an indication of their common source from *Fusarium* sp. The results suggest low levels and safe exposure to consumers of Kenyan lager beers with *Fusarium* mycotoxins.

Key words: Aflatoxins, *Fusarium* mycotoxins, Kenyan lager beers.

INTRODUCTION

Cereals used in beer production are prone to mycotoxin contamination due to the presence of toxicogenic molds. This is particularly common with naked cereal grains such as maize, wheat, sorghum and millet, which unlike barley, are not protected by the presence of husks. Naturally occurring molds grow easily on these grains during malting or high moisture storage conditions. The growth of molds such as *Aspergillus flavus*, *Penicillium parasiticus*, *Fusarium graminearum*, *F. culmorum*, *F. roseum* and *F. moniliforme* on grains or during malting are known to elaborate aflatoxins, trichothecenes, fumonisins, ochratoxin A and zearalenones, among other mycotoxins^{1,2,9,14}. Schwarz et al.⁹ for example reported an increase of deoxynivalenol (DON) by 18–114% of that present on the original barley in 5 day green malts, attributable to the level of infestation

on barley viability and growth by *F. graminearum*. Use of adjuncts contaminated with these mycotoxins can also lead to possible contamination of the finished beers. These mycotoxins are said to survive the major beer production processes namely malting, mashing, boiling and fermentation^{9,10}. After brewing, 80–93% of DON present on the malt grist was detected in the beer according to Schwarz et al.⁹, indicating a high extraction rate of DON from spent grains during mashing, due to its water solubility. Previous studies in Kenya have reported high incidences and levels of mycotoxin contamination in cereals, cereal-based foods and feeds as well as in the traditional commercial opaque beers^{5,7,8}. Consumption of DON, also known as vomitoxin, causes a range of symptoms including abdominal pain, dizziness, headache, throat irritation, nausea, vomiting, and diarrhea. Both in vitro and in vivo studies on the toxicity and interaction effects by mixture of *Fusarium* mycotoxins, and in particular trichothecenes, have pointed to the possibility of toxicity exhibition on the basis of dose additivity, antagonistic or synergistic responses¹.

This paper reports data on the incidences and levels of four mycotoxins, namely aflatoxin B₁ (AFB₁), deoxynivalenol (DON), fumonisin B₁ (FB₁) and zearalenone (ZEA), in two brands of Kenyan lager beers.

MATERIALS AND METHODS

Sampling

A total of 75 samples of bottled commercial beer were randomly collected from bars in the city of Nairobi and the satellite towns. Of the 75 samples, 39 were Tusker and 36 were Pilsner lager beers.

Extraction

Each bottled beer sample was gently shaken and approximately 100 mL of beer drawn. The 100 mL beer was degassed using a vacuum pump and subjected to solid-phase extraction using C₁₈ extraction columns (Isolute® – International Sorbent Technology Ltd. UK), mounted on a solid-phase extraction manifold (Vacmaster®). The C₁₈ columns were equilibrated by passing 10 mL of methanol:water (10:90) solvent. The degassed beer (20 mL) was then passed through the column and the column washed by passing through 20 mL of distilled water, followed by drying with air. The mycotoxins in the column were eluted with 2 mL of methanol, which was diluted 1:10 with phosphate buffered saline (PBS) solution, before analysis by the ELISA procedure.

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Table I. The incidence of mycotoxins in the lager beers.

Mycotoxin	Tusker (n=39)	Pilsner (n = 36)	Both (n = 75)
DON	39/39 (100%)	36/36 (100%)	75/75 (100%)
Fumonisin B ₁	30/39 (76.9%)	24/36 (66.7%)	54/75 (72%)
Zearalenone	39/39 (100%)	36/36 (100%)	75/75 (100%)

Table II. The levels of mycotoxins in the two brands of lager beers.

Mycotoxin	Brand	N	Mean	Minimum	Maximum
DON (ng/mL)	Tusker	39	3.29 ± 1.15	1.56	6.40
	Pilsner	36	3.57 ± 0.66	2.40	4.35
	Total	75	3.42 ± 0.96	1.56	6.40
Fumonisin B ₁ (ng/mL)	Tusker	39	0.28 ± 0.18	0.00	0.49
	Pilsner	36	0.32 ± 0.26	0.00	0.78
	Total	75	0.30 ± 0.22	0.00	0.78
Zearalenone (pg/mL)	Tusker	39	7.84 ± 1.19	5.70	10.10
	Pilsner	36	8.50 ± 1.66	4.30	10.20
	Total	75	8.16 ± 1.46	4.30	10.20

N = Number of samples.

Mycotoxin analysis

All analyses were performed using direct competitive microplate enzyme-linked immunosorbent assays (ELISA). These assays were performed as previously described for FB₁¹⁴, AFB₁³, and DON and ZEA¹³. The microtitre plate wells (Maxisorp[®] Nunc, Denmark), were coated with anti-mycotoxin antibody solution in 0.1M sodium bicarbonate buffer and incubated overnight at room temperature. Free protein binding sites were blocked with 3% fetal calf serum in phosphate buffer solution (200 µL/well) for 30 min. The wells were then washed three times with NaCl-Tween (8.5 g NaCl and 250 µL of Tween-20 in 1 liter of water) solution. Aliquots (50 µL) of diluted beer extracts or respective mycotoxin standards were added into the well, followed by addition of aliquots (50 µL) of the respective mycotoxin horse radish peroxidase conjugate. The plates were incubated for two hours at room temperature, washed, and an enzyme substrate solution (100 µL) added. The enzyme reaction was stopped by addition of 1M sulfuric acid (100 µL) and absorbance read at 450 nm using an ELISA reader (Uniskanii[®] LabSystems, Finland). Absorbance values were analyzed with a competitive ELISA software⁶, and the data statistically analyzed for variability and association using the SPSS version 10.

RESULTS AND DISCUSSION

The ELISA assay technique has a detection limit of 1.045, 0.201, 0.032 and 0.085 parts per billion (ppb), for DON, FB₁, ZEA and AFB₁ respectively.

Data from Table I showed 100% incidences for DON and ZEA in both brands. However, only 72% of the beer samples were positive for FB₁, while all the samples were negative for AFB₁. The percentage incidences of DON for beer samples analyzed in France, Canada, Germany, USA and Spain ranged from 0.02 to 100%^{4,10,12}. However, six studies on mycotoxins contamination of beers in U.S.A, Canada and Europe, using thin layer chromatographic techniques, reported DON as absent¹⁰. Scott¹⁰ reported further that gushing beer is significantly associated with

higher levels of DON, compared with non-gushing beer. Zearalenone was negative in European and Canadian beers, while Fumonisin was detected in 7 out of 30 beers produced in Canada, and 2 out of 11 imported beers¹⁰. Out of 54 samples of Korean beers, none was found to contain ZEA¹¹. Previous studies by Mbugua and Mwaura⁷ reported presence of AFB₁ in Busaa, a local traditional and commercial opaque beer in Kenya, using thin layer chromatographic assays, but did not detect any in the lager beers. With detection range limits of 0.14–10 ppb for thin layer chromatographic method, European beers detected negative as well, for aflatoxins. However with newer sensitive immunoaffinity column methods with a detection range of less than 0.1 ng/mL, aflatoxins B₁ was detected in 3 beers imported into the U.K. from Mexico¹⁰. In Japan 12.5 ng/litre of aflatoxin B₁ in beer were also reported⁹. Absence of *Fusarium* mycotoxins in lager beers is an indication of either absence of *Fusarium* fungus on the malts used, or effective screening of the fungus by the malting industry.

Data in Table II show the mean levels for DON as 3.29 and 3.57 ng/mL in Tusker and Pilsner respectively. The levels ranged from 1.56–6.40 ng/mL. The highest level for DON was in Tusker beer, although there was no significant difference ($p < 0.05$) between the means for the two brands. The mean levels for Fumonisin B₁ were 0.28 and 0.32 ng/mL in Tusker and Pilsner respectively, and ranged from nondetectable levels to 0.78 ng/mL, this time in Pilsner. However, there was no significant difference ($p < 0.05$), between the mean values for the two brands. These levels were very low, and can be partially attributed to the use of corn starch rather than corn grits as adjuncts. Fumonisin have not been detected in starch produced by the wet milling process¹⁰. The mean values for zearalenone were 7.84 and 8.50 pg/mL in Tusker and Pilsner respectively, and ranged from 4.30 to 10.20 pg/mL. The highest levels (10.2 pg/mL) were in Pilsner, where the mean values were significantly higher ($p < 0.05$) than in Tusker. The ZEA levels in the two brands were very low. Other studies have established that ZEA are found in much lower concentrations than DON in grains including

Table III. Pearson correlation coefficients of the mycotoxins in two beer brands.

	DON	Fumonisin B ₁	Zearalenone
DON	1.000	0.642**	0.248*
Fumonisin B ₁	0.642**	1.000	0.120
Zearalenone	0.248*	0.120	1.000

**Correlation is significant at $P < 0.01$ (2-tailed).

*Correlation is significant at $P < 0.05$ (2-tailed).

barley¹⁰. Furthermore, ZEA is said to be converted to β -zearalenol in the order of 63–72%, and to a lesser extent α -zearalenol (3%) by the brewing yeast, while other studies report low extraction from contaminated spent grains into the wort and finally into the finished beer¹⁰. Most of the European beers tested, that contained DON had an average of 5–20 ng/mL, with the exception of some German wheat beer samples, which had up to 570 ng/mL¹⁰.

The data in Table III show that a positive association exists between DON and FB₁, as well as DON and ZEA. This means that if DON was present, chances were that FB₁ and ZEA were also likely to be present. This can be both theoretically and practically expected, since the three mycotoxins are elaborated by *Fusarium* fungi which commonly contaminate maize and other cereals.

Toxicity limits and implications

European Commission has tentatively set tolerable daily intake (TDI) for DON at 1 mg/kg body weight per day¹. For a normal man weighing 60 kg, this means consumption of 60 mg DON per day. At the maximum level of 3.2 mg per 500 mL bottle (6.4 ng/mL) in Tusker, this would imply consumption in excess of 20 bottles per day, which is both abnormal and highly unlikely. According to the U.S. food and drug administration¹⁵, there is no direct evidence on FB₁ intake levels that have adverse health effects in humans. Toxicity in rats and poultry involves kidney and liver tumors, while in horses involves hyperexcitability, incoordination, stupidity and depression¹⁵. The maximum levels allowed in maize products, commonly contaminated with FB₁, range from 2–4 ppm¹⁵. This means that the maximum level of FB₁ (0.78 ng/mL) found in Pilsner was about 2,000 times less than 2 ppm, the lower maximum level recommended for maize products. Accordingly such levels are unlikely to be ingested by consumption of Pilsner. The average TDI for ZEA in pigs, the most sensitive animal to ZEA toxicity is 0.06 μ g/kg body weight per day². Again no consumer is likely to ingest ZEA levels anywhere near this level by drinking Pilsner, given the maximum levels of 10.2 pg/mL. Some concern however can be raised regarding the potential toxicity implication, when beer is contaminated by mixtures of these mycotoxins as suggested in this study. The reported potential toxicity interaction by mixtures of the mycotoxins in their low concentrations¹, that is whether additive, antagonistic or synergistic, should be a subject of future investigation, especially when ingested in the presence of alcohol, as the liver in humans is the main detoxification organ.

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