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Short Communication

Deoxynivanol (DON) and fumonisins B_1 (FB_1) in artisanal sorghum opaque beer brewed in north Cameroon

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The production and consumption of artisanal home-brewed sorghum beer (*Bil-bil* and *Kpata*) is a widespread traditional practice in the northern Sudan-Sahelian zone of Cameroon. Deoxynivanol (DON) and fumonisins B_1 (FB_1) in these home-brewed artisanal opaque beer samples extracted by HPLC method, before analysis by the enzyme-linked immunosorbent assays (ELISA) procedure, showed a wide range of levels. All samples were positive for DON and FB_1. DON levels varies from 140 to 730 ng/ml with a mean of 450 ±90 ng/ml for *Bil bil* and from 0.0 to 680 ng/ml with a mean of 520±70 ng/ml in *Kpata*. The recorded levels of fumonisin B_1 varies from 0.0 to 230 ng/ml with a mean of 150±24 ng/ml in *Bil-bil* and from 0.5 to 340 ng/ml with a mean of 210±10 in *Kpata*. On the basis of published data for the consumption of artisanal home-brewed sorghum beer (*Bil-bil* and *Kpata*) in Cameroon, the fumonisin and deoxynevanol exposure in these regions among the consumers was found to be well above the provisional maximum tolerable daily intake.

Key words: Fumonisins, mycotoxins, North Cameroon, beer, sorghum, *Fusarium.*

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench), the fifth most widely produced crop in the world, is a traditional cereal crop for millions of people in Cameroon. In this country, the production of sorghum is practised especially in the Sudan-Sahelian zone on approximately 300.000 ha for an average production of 100.000 tons (Djamen et al., 2003). Mainly the sorghum grain is used in the preparation of several national diets and mostly in the making of local beers named "*Bil-bil* "and "*Kpata*" (Djoulde et al., 2008). It's generally known that naked cereal grains such as sorghum and millet which are not protected by the presence of husks used in beer production are prone to mycotoxin contamination due to the presence of toxinogenic moulds (Schwarz et al., 1995). Moreover, previous studies in Cameroon have reported high incidences and levels of mycotoxin contamination in cereals, cereal based foods and feeds (Ngoko et al., 2008). This paper thus aims at highlighting the incidences and levels of mycotoxins, in artisanal sorghum beer produced in northern Cameroon.

MATERIALS AND METHODS

Description of samples

Bil-bil is an artisanal opaque beer made of 3 days humid malted sorghum grains mixed with sorghum flour, moisture and fermented for 24 h by an artisanal starter culture made of a mixture of an unidentified yeast, mould and bacteria. *Kpata is made in the same way as Bil-bil* except that sorghum flour is replaced by corn flour.

Sampling

A total of 120 samples of two locally produced artisanal home-brewed sorghum opaque beers namely *Bil-bil* (70) and *Kpata* (50) were randomly collected from traditional breweries in the cities of Garoua, Maroua and Ngaoundere in the northern part of Cameroon.

Mycotoxins extraction

Approximately 100 ml per sample of opaque beer were gently shaken and degassed using a vacuum pump. The degassed

Table 1. Incidence and levels of DON and FB1 in sorghum opaque beer from north Cameroon.

Mycotoxins	Bil- bil (n=70)		Kpata (n=50)	
	Incidence	Levels (ng/ml) (min- max)	incidence	Levels (ng/ml)(min- max)
DON	70/70 (100%)	140-730	37/50 (74%)	0.0-680
Fumonisins B_1	55/70 (78.5%)	0.0-230	50/50 (100%)	0.5-340

opaque beer were then subjected to solid phase extraction using extraction columns C_{18} (Perkin Elmer, Norwalk, USA), mounted on a solid-phase extraction manifold (Vacmaster®). The C_{18} 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 enzyme-linked immunosorbent assays (ELISA) procedure.

Mycotoxin analysis

All analyses were performed using direct competitive microplate enzyme-linked immunosorbent assays (ELISA) as previously described by Usleber et al. (1992, 1994). The microtitre plate wells were coated with antimycotoxin antibody solution in 0.1 M sodium bicarbonate buffer and incubated 18 h at room temperature. The wells were then washed three times with NaCl-Tween (8.5 g NaCl and 250 µl of Tween-20 in 1 L of water) solution after the free protein binding sites were coated with 3% fetal calf serum in phosphate buffer solution (200 µl/well) for 20 min. Aliquots (50 µl) of diluted beer extracts and 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 absorbances were read at 450 nm using an ELISA reader reaction after the reaction was stopped by addition of 1 M sulfuric acid (100 µl). Absorbance values were analysed with a competitive ELISA software (Märtlbauer, 1993), and the data statistically analysed for variability and association using the SPlus 2000.

RESULTS

The ELISA assay technique has a detection limit of 1.045 for DON and 0.201 for FB1. Data from Table 1 showed 100% incidences for DON in *Bil-bil* and for FB1 in *Kpata*. We also recorded 78.5% incidence for FB1 in *Bil-bil* and 74% for DON in *Kpata*.

The level of DON goes from 140 to 730 with a mean of 450 ±9 ng/ml for *Bil bil* and from 0.0 to 680 ng/ml with a mean of 520±70 in *Kpata*. The recorded levels of fumonisin B_1 varies from 0.0 to 230 ng/ml with a mean of 150±24 ng/ml in *Bil-bil* and from 0.5 to 340 ng/ml with a mean of 210±10 in *Kpata* (Table 1).

Although there is no significant difference between levels of mycotoxins in *Bil-bil* and *Kpata*, we notice a light increase in *Kpata* opaque beer than *Bil- bil* (Table 1). This can be partially attributed to the use of maize rather than sorghum grits as adjuncts in *Kpata* process production (Ngoko et al., 2008). There seems to be a positive association between DON and FB1. This can be both theoretically and practically expected, since the two mycotoxins are elaborated by *Fusarium* fungi which commonly contaminate maize and sorghum witch are the main ingredient for the two opaque beer production.

DISCUSSION

The percentage incidences of DON for beer samples analysed in other developed countries ranged from 0.02 to 100% (Hlywka and Bullerman, 1999; Torres et al., 1998). The presence of DON and FB_1 in those opaque beers is an indication of presence of mould on the malts used. Naturally occurring moulds grow easily on sorghum grains during malting or high moisture storage conditions which are the main stage of African opaque beer process production. The growth of moulds such as *Aspergillus flavus, Penicillium parasiticus, Fusarium graminearum, Fusarium culmorum, Fusarium roseum* and *Fusarium moniliforme* on grains or during malting are known to elaborate aflatoxins, trichothecenes, fumonisins, Ochratoxin A and zearalenones, among other mycotoxins (Reza et al., 2007; Tores et al., 1998). Schwarz et al. (1995) recover 80 to 93% of DON present on the malt grist after brewing, indicating a high extraction rate of DON from spent grains during mashing. This can be directly linked to DON water solubility. In fact many mycotoxins seems to survive major African opaque beer production processes namely malting, mashing, boiling and fermentation (Scott, 1996). 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 responses (European Commission, 2002).

European Commission has tentatively set tolerable daily intake (TDI) for DON at 1 mg/kg body weight per day. For a normal man weighing 60 kg, this means consumption of 60 mg DON per day. If we consider that one measure (earthen jar) equal to about 500 ml, at the maximum level of 730 ng/ml DON in *Bil-bil*, this would imply consumption of 15 earthen jar per day, which is both abnormal and highly unlikely. The same considerations are for fumonisin. If we consider the fact that, there is no direct evidence on FB_1 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

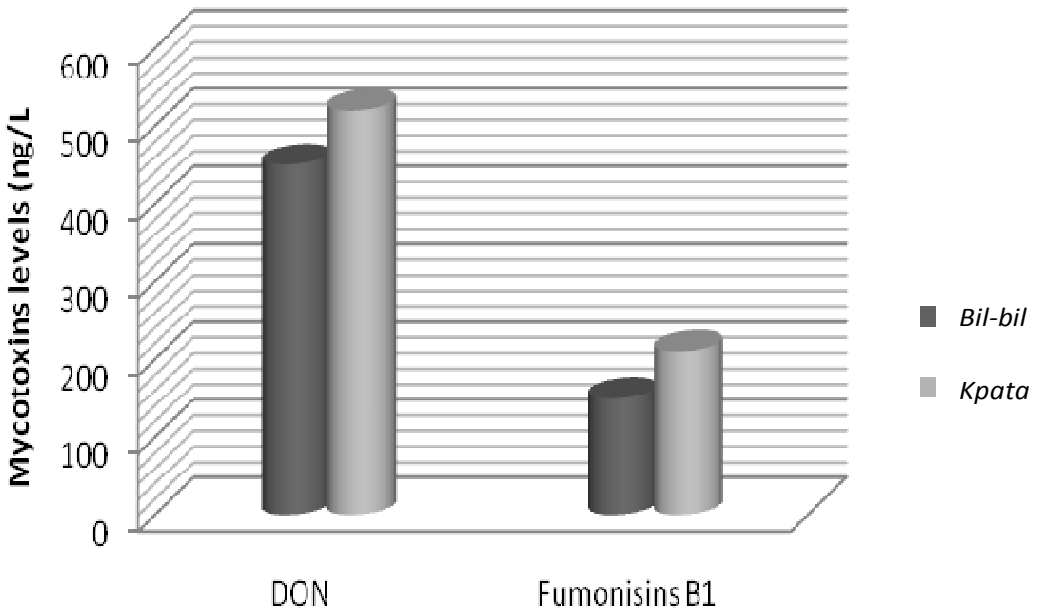

Figure 1. DON and FB1 levels in *Bil-bil* and *Kpata*, two Cameroonian artisanal opaque beer.

depression.

The maximum levels allowed in maize products, commonly contaminated with FB_1, range from 2 to 4 ppm. This means that the maximum level of FB1 (680 ng/ml) found in *Bil-bil* was about 200 times less than 2 ppm, the lower maximum level recommended for maize products. Accordingly such levels are unlikely to be ingested by consumption of *Bil-bil.*

Conclusion

High levels of DON and FB_1 were recorded in *Bil-bil* and *Kpata*, two Cameroonian artisanal opaque beers Figure 1. If we consider the fact that, there is no direct evidence on their intake levels that have adverse health effects in humans, some concern however can be raised regarding the potential toxicity implication, when contaminated by mixtures of these mycotoxins as suggested in this study.

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