

## Research Article

# Occurrence and Exposure Assessment of Aflatoxin B<sub>1</sub> in Omena (*Rastrineobola argentea*) from Kenya

Esther Marijani <sup>1</sup>, Harrison Charo-Karisa,<sup>2</sup> Emmanuel Kigadye,<sup>1</sup> and Sheila Okoth<sup>3</sup>

<sup>1</sup>Open University of Tanzania, P.O. Box 23409, Dar es Salaam, Tanzania

<sup>2</sup>State Department of Fisheries and the Blue Economy, Fisheries Resources Development and Marketing, P.O. Box 58187, Nairobi, Kenya

<sup>3</sup>University of Nairobi, School of Biological Science, P.O. Box 30197-00100, Nairobi, Kenya

Correspondence should be addressed to Esther Marijani; [esthermarijani@gmail.com](mailto:esthermarijani@gmail.com)

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Omena (*Rastrineobola argentea*) is the most consumed fish species in Kenya. In this study, we assessed the occurrence of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in Omena and the potential health risk of AFB<sub>1</sub> to Kenyan consumers of this fish. A total of 74 samples comprising Omena intended for human consumption and fish feed production were analyzed in this study. Aflatoxin levels in Omena were determined using the enzyme-linked immunosorbent assay (ELISA). Omena intended for fish feed production was most contaminated with a mean concentration of 46.93  $\mu\text{g}\cdot\text{kg}^{-1}$  (2.24–115.23  $\mu\text{g}\cdot\text{kg}^{-1}$ ) compared with Omena intended for human consumption (mean = 19.42  $\mu\text{g}\cdot\text{kg}^{-1}$ , range = 2.01–49.30  $\mu\text{g}\cdot\text{kg}^{-1}$ ). Thirty-five positive samples (83.3%) exceeded the maximum level permitted (5  $\mu\text{g}\cdot\text{kg}^{-1}$ ) by the East Africa Community standard for food used for human consumption. The exposure dose of AFB<sub>1</sub> from consuming Omena was estimated to be 1.34  $\text{ng}\cdot\text{kg}^{-1}\text{ BW day}^{-1}$ , and margin of exposure (MoE) value for AFB<sub>1</sub> was found to be 126.3, which indicates health risk to Omena consumers. The results suggest that the current situation of aflatoxin contamination in Omena has an adverse effect on the health of the consumers as well as the animals. Therefore, more surveys are needed to understand the scope and extent of aflatoxin contamination in Omena.

## 1. Introduction

Fish is an important part of nutrition, contributing on average 22% of animal protein intake in the East and Central African regions [1]. Fish and fish products provide vital vitamins, minerals, fatty acids, and other micronutrients crucial to a healthy diet [2]. The most common fish species consumed in Kenya are tilapia and *Rastrineobola argentea* (locally known as “Omena” or “Dagaa”). Other species include Nile perch (locally known as “Mbuta”) and catfish [1]. According to the FAO [3], the production of Omena, tilapia, Nile perch, and catfish in Kenya was 69,561, 47,555, 43,399, and 11,398, respectively, in 2016.

Fish consumption rates are increasing due to fast-growing population and awareness of the health benefits associated with consuming fish, as well as rising

urbanization [1]. Fish consumption in Kenya is estimated at 4.5 kg per capita/year [4]. Omena is the most important small fish species that contributes immensely to the protein needs of the poor people [5], accounting for 35% of the country’s total fish human consumption. Between 60 and 70% of Omena is processed as animal feed [4]. However, poor handling, processing, and packaging methods and practices are used in Omena operations. The traditional practice of drying fish on the ground or on old fishing nets is still common in Kenya [5]. This practice results in pathogenic contamination of fish products, such as sun-dried Omena, and smoked catfish by fungi [6–8]. Over an extended period of time, these pathogenic fungi can multiply and their metabolites cause changes in the feed and food quality that can adversely affect the health of animals and humans [9].

Aflatoxins (AFs) are mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*, which grow on numerous food and feedstuffs when environmental conditions are favourable [10]. There are four common types of aflatoxins: AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub>. AFB<sub>1</sub> is the most potent, among them, to both humans and animals [11]. Aflatoxins can cause acute poisoning and mortality to humans and animals, usually due to liver cirrhosis. Also, inhalation or absorption of chronic lower-level doses of aflatoxins through the skin can result in cancer of the liver and chronic immunosuppression [12, 13]. All doses have a cumulative effect on increasing the risk of cancer. Cases of aflatoxicosis outbreak have occurred in Kenya after consuming maize, which are highly contaminated by aflatoxins [14]. Due to these outbreaks, aflatoxin contamination studies have focused on maize and maize products, milk, and groundnuts [15–20]. Contamination of fish in Kenya with aflatoxins has been largely ignored. There are few studies on aflatoxin contamination of Omena intended for either human or animal consumption [6, 7]. These studies report a low level of aflatoxin contamination (0 and 0.33–1.58  $\mu\text{g}\cdot\text{kg}^{-1}$ ) in dried Omena collected from the outskirts of the main city, Kisumu, in the Nyanza region. Hence, the present study evaluates the occurrence of aflatoxins in Omena intended for both human and fish feed production collected in Kisumu city. We further evaluate possible human exposure to aflatoxins as a result of the consumption of Omena.

## 2. Materials and Methods

**2.1. Sample Collection.** A total of 42 samples from 21 vendors were collected randomly from Kibuye Market, (Kisumu City). Two 1 kg samples of Omena from each vendor were collected by mixing thoroughly the lower and upper layers and different angles of Omena in the vendor's table (Figure 1). A 100 g subsample was used for analysis after some further mixing at the laboratory. Omena used as fish feed ingredients ( $n = 32$ ) were collected from sixteen farmers who processed their own feed at the farm level from the County. Collected samples were transferred to the mycology laboratory, University of Nairobi, under complete aseptic conditions. Samples were finely ground using a Romer Mill (Romer series II® MILL) and thoroughly mixed before aflatoxin analysis.

**2.2. Aflatoxin Quantification.** Aflatoxins were extracted from five grams of ground samples with 25 mL of methanol: deionized water (70 : 30 v/v) (Thermo Scientific, USA). Then, the extract was mixed vigorously on a magnetic stirrer for 3 minutes. The extract was filtered through a Whatman No. 1 filter paper (from Sigma-Aldrich, St Louis, MO, USA) and diluted 1:10 into phosphate-buffered saline (PBS). The extracts were assayed for AFB<sub>1</sub> using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol (Helica Biosystems Inc., Santa Ana, CA).



FIGURE 1: Omena used in the current study as collected from markets in Kisumu City, Kenya.

Absorbance at 490 nm was recorded using an ELISA analyzer (BioTek Instruments, Inc., VT, USA). The content of AFB<sub>1</sub> in samples was calculated by using ReaderFit software (Hitachi Solutions America Ltd, San Francisco, CA). Sample concentrations below the LOD were reported as not detected (n.d.). Sample concentrations that exceeded 20 ng/g (the highest concentration of aflatoxin standard) were further diluted. The diluted sample concentration was multiplied by the dilution factor to obtain the actual concentration of AFB<sub>1</sub> in the original sample. All samples were analyzed in triplicate to obtain mean concentrations and standard deviations.

**2.3. Method Validation.** The ELISA method was validated through sensitivity, linearity, accuracy, and precision parameters. The linearity of the calibration curve was assessed by calculating linear regression and coefficient of determination (R<sup>2</sup>), using five points of the standard curve.

Limit of detection (LOD) was used to determine the sensitivity of the method. LOD was determined by calculating the mean concentration of 18 blank matrix samples plus three standard deviations, while the limit of quantification (LOQ) was the mean value plus ten standard deviations.

Accuracy was assessed by assaying the recovery of AFB<sub>1</sub>, by spiking 2.5, 5, 10, and 20  $\mu\text{g}\cdot\text{kg}^{-1}$  of AFB<sub>1</sub> of the uncontaminated samples. Percent recovery was calculated by taking the difference of the amount of AFB<sub>1</sub> spiked into the sample and the amount of AFB<sub>1</sub> recovered from the assay divided by the amount of AFB<sub>1</sub> spiked into the sample multiplied by 100%.

The coefficient of variation (CV) of intraplate and interbatch was used to determine the precision of the method. The CV of intraplate was calculated as the ratio of the standard deviation to the mean of six parallel microwells in the same plate at each AFB<sub>1</sub> level. The ratio of the standard deviation to the mean of two plates at each AFB<sub>1</sub> level was used to calculate the CV of interplate.

**2.4. Dietary Exposure of AFB<sub>1</sub>.** AFB<sub>1</sub> exposure assessment was determined based on mean body weight of Kenyans (60 kg) [21] and Omena consumers' mean consumption of

the fish as reported by Farm Africa [4], Jumbe et al. [5], and Kariuki [22].

The AFB<sub>1</sub> daily intake was calculated as follows:

- (1) Estimated daily intake (EDI) = daily intake (Omena) × means level of AFB<sub>1</sub>/body weight (kg), where EDI is expressed in  $\mu\text{g}\cdot\text{kg}^{-1}$  of bodyweight/day ( $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ ).
- (2) The dietary exposure to AFB<sub>1</sub> at 95th percentile =  $(L \times D)/\text{BW}$  (kg), where  $L$  is the 95th percentile concentration of aflatoxin in the samples and  $D$  is the daily consumption of Omena ( $\text{g}\cdot\text{person}^{-1}\cdot\text{day}^{-1}$ ). For calculation of AFB<sub>1</sub> intake, the value was assumed to be 0 when the result was below the limit of detection.

The estimated potency of liver cancer was determined using the Joint FAO/WHO Expert Committee on Food Additives model [23]. According to the FAO/WHO, the population risk for primary liver cancer can be estimated with an assumption of 25% carriers of hepatitis B in developing countries [23]. The potencies of hepatitis B virus (HBV) infection and HBV noninfection values are 0.3 and 0.01, respectively, estimated from animal and epidemiological studies [23]. Hence, the potency of liver cancer in the Kenyan population can be estimated using the following equation:

$$\text{average potency (cancer cases/year/100,000 people)} = (0.01 \times 75\%) + (0.30 \times 25\%) \times \text{AFB}_1 \text{ intake (ng} \cdot \text{kg}^{-1} \cdot \text{BW} \cdot \text{day}^{-1}). \quad (1)$$

**2.5. Risk Assessment.** The margin of exposure (MoE) method estimates the risk of genotoxic carcinogens [24]. MoE calculates the risk by the ratio of carcinogenic dose (or population carcinogenic dose) to population intake.

In this study, the MoE was calculated by dividing the benchmark dose lower limit (BMDL) by the EDI of AFB<sub>1</sub>.  $\text{BMDL}_{10}/\text{exposure}$ , where BMDL<sub>10</sub> is the benchmark dose lower confidence limit of 10% of  $170 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ , was proposed by the European Food Safety Authority [24].

**2.6. Data Analysis.** The descriptive analyses of mean and standard errors were performed with Statistical Package SPSS v21 (IBM Corporation, Armonk, NY, USA). Statistical analysis was done using one-way analysis of variance (ANOVA) with the Bonferroni test at the 5% level of significance to determine significant differences in the levels of AFB<sub>1</sub> between groups.

### 3. Result and Discussion

**3.1. Method Validation.** Coefficients of variation of interplate and intraplate ranged between 9.07% and 12.71% and between 1.01% and 2.58%, respectively (Table 1). The recovery of AFB<sub>1</sub> from all spiked samples was 92% to 109% (Table 1). These results were in accordance with the European Commission (EC) Regulation No. 401/2006 that establishes recoveries in the range of 70–110% and 50–120%

TABLE 1: Validation data of the ELISA for AFB<sub>1</sub>.

AFB <sub>1</sub> spiked	AFB <sub>1</sub> found	Recovery (%)	Coefficient of variation	
			Intraplate	Interplate
2.5	2.28	92	1.3	9.07
5	5.46	109	1.01	12.71
10	9.80	98.8	1.11	11.65
20	19.53	97.7	2.58	12.54

[25]. The LOD and LOQ values were estimated to be 0.81 and  $2.4 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively.

**3.2. Aflatoxin Contamination in Omena Samples.** Aflatoxins are a threat to human and animal health, animal productivity, and trade [26–29]. In the current study, the result shows that Omena intended for human food are contaminated with aflatoxin with a maximum concentration of  $49.30 \mu\text{g}\cdot\text{kg}^{-1}$  (Table 2). Also, more than eighty percent of the Omena for human food was contaminated with AFB<sub>1</sub> above the acceptable limits of  $5 \mu\text{g}\cdot\text{kg}^{-1}$  as recommended by the East Africa Community [30]. Our results are higher than those of a previous study by Orony et al. [31] who found out that Omena from Kenya were contaminated with aflatoxins with a mean concentration of  $0.33\text{--}1.58 \mu\text{g}\cdot\text{kg}^{-1}$ . However, another study from Kenya reported that aflatoxins were not detected from Omena samples [6]. *Aspergillus flavus*, which is an aflatoxin producer, was detected from Omena samples from Kenya [7], in a study that, although did not analyze the presence of aflatoxin, concluded that the presence of aflatoxin-producing species in the samples indicates that the Omena could be a threat to the health of the consumers [7]. The present study found higher aflatoxin contamination in Omena compared with previous studies on dried fish and smoked fish from Zambia and Nigeria [32–34]. Dried fish (*Oreochromis*, *Petrocephalus*, and *Limnothrissa* genera) from Zambia were contaminated with the mean concentration of  $>2.9$  and  $5 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively [33]. Adebayo et al. [32] found aflatoxin contamination ranging from 1.5 to  $8.1 \mu\text{g}\cdot\text{kg}^{-1}$  in dried fish and 2.7 to  $4.0 \mu\text{g}\cdot\text{kg}^{-1}$  in smoked dried fish from Nigeria [8]. Smoked dried fish from Nigeria were contaminated with AFB<sub>1</sub> ranging from 2.731 to  $4.031 \mu\text{g}\cdot\text{kg}^{-1}$  [8]. Olajuyigbe et al. [34] reported that dried finfish and shellfish from Nigeria were contaminated with a mean aflatoxin concentration of 5.4 and  $5.2 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively [34].

After Omena is harvested from Lake Victoria, it is traded and processed mostly by women who derive their livelihood from this trade [5]. At harvest, Omena are considered free of aflatoxin contamination. However, aflatoxin accumulation may occur during processing, transport, or poor storage. Majority of fish processors sun-dry the fish on fishing nets spread on the ground or directly on the ground [5]. Often, Omena are transported over long distances to various destinations for a long time under suboptimal conditions of heat and humidity, which provides favourable conditions for the growth of pathogenic fungi. The high aflatoxin contamination of Omena observed in the present study may be

TABLE 2: Aflatoxin levels in Omena for human and fish feed production and risk assessment due to consumption of Omena.

Samples	N	Range ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	Mean $\pm$ SEM ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	% samples $>20 \mu\text{g}\cdot\text{kg}^{-1}$	% samples $>5 \mu\text{g}\cdot\text{kg}^{-1}$	EDI ( $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ )		Population risk for primary liver cancer (cancer/year/ 100,000 population)	
						Mean	P95	Mean	P95
Omena for human consumption	42 (41)	2.01–49.30	$19.42 \pm 1.96^a$	38.00	83.33	1.35	2.78	0.11	0.22
Omena for fish feed production	32 (31)	2.24–115.23	$46.93 \pm 6.17^b$	75.00	93.75				
Total	74 (72)								

Values provided in parentheses under column N indicate the number of positive samples above the limit of detection (LOD); a, b = mean values followed by similar letter do not differ significantly ( $P < 0.05$ ). BMDL10/exposure, where BMDL10 is the benchmark dose lower confidence limit of 10% of  $170 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ , proposed by the European Food Safety Authority [24]. MoE: margin of exposure; EDI: estimated daily intake.

attributed to these practices, especially poor storage and improper drying. Another reason for high contamination observed in the present study might be because the samples were collected during the rainy season, which increases the chances of fungal growth and aflatoxin production.

The results in the present study show a significant difference ( $P < 0.05$ ) in aflatoxin concentration among Omena samples intended for human consumption and fish feed production (Table 2). Omena for fish feed production were the most contaminated samples ranging from 2.24 to 115.23  $\mu\text{g}\cdot\text{kg}^{-1}$  compared with Omena for human consumption (2.01–49.30  $\mu\text{g}\cdot\text{kg}^{-1}$ ) (Table 2). This might be attributed to the fact that Omena used for animal consumption is usually rejected for human consumption because of poor quality. The presence of debris and sand, wetness, and discoloration in the Omena often leads to its rejection as human food and instead gets used to produce animal feed. Such conditions like wetness promote aflatoxin production by toxigenic fungi [7], thus confirming our result which shows that Omena for feed production were more contaminated with aflatoxin compared with those for human food. All samples collected during the rainy season when temperature and humidity were 32°C and 78%, respectively, together with poor storage practices and improper handling increases the risk of aflatoxin contamination [5–7].

More than seventy percent of the fish for feed production was above acceptable limits of 20  $\mu\text{g}\cdot\text{kg}^{-1}$  recommended by the East Africa Community [30]. Our previous study shows that ingredients used in fish feeds were contaminated by aflatoxins up to 806  $\mu\text{g}\cdot\text{kg}^{-1}$  [35]. Sunflower, maize bran, and cottonseed cake were highly contaminated by aflatoxin [35]. Since aflatoxins affect fish health, which can result in low production, it is important to routinely monitor raw materials as well as finished feeds.

**3.3. Dietary Exposure of AFB<sub>1</sub>.** We used the EDI approach to determine exposure to AFB<sub>1</sub>. Several studies using the estimation of EDI values on AF exposure in Omena, maize, and peanuts have been reported in Africa [14, 31, 36–38]. At 1.34  $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$  and the 95th percentile of exposure (high consumer) at 2.78  $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$  (Table 2), the estimated mean AFB<sub>1</sub> exposure obtained from the present study was much higher than those reported in Ghana and Nigeria, with the mean dietary intake of aflatoxin in rice and peanuts (0.013 and 0.17  $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ ), respectively [39, 40]. Similarly, the estimated daily intake of aflatoxins through the consumption of Omena from this study was higher than that of Orony et al. [31]. Strikingly, our results were lower than aflatoxin exposure previously reported in maize from Kenya (292  $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ ) [14]. This might be attributed to the fact that daily consumption of maize is higher than that of Omena, which exposes maize consumers to a higher risk of aflatoxin contamination.

Nevertheless, the estimated exposure levels of AFB<sub>1</sub> for the Kenyan population from Omena consumption are high enough to cause public health concerns due to the fact that even low levels of AF contamination (1  $\text{ng}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{day}^{-1}$ )

may induce liver cancer cases [41]. The potency of liver cancer in Kenya is 0.11 cancer cases/year/100,000 people for average Omena consumption, while at 95th percentile consumption; the potency of liver cancer was two times higher (Table 2). In Kenya, cases of liver cancer have been increasing with an age standardized incidence rates (ASR) of 7.2 per 100,000 [42]. Therefore, it can be estimated from our results that consuming aflatoxin-contaminated Omena could be responsible for 1.52% of all cancer cases in Kenya (0.11/7.2 × 100). This should not be ignored since there is a greater contribution that may take place for the population consuming a daily amount of Omena above the national average.

The margin of exposure (MoE) approach was applied to characterize the risk of consuming Omena contaminated with AFB<sub>1</sub>. Several studies previously used the margin of exposure (MoE) approach for risk characterization of genotoxic and carcinogenic mycotoxins like AFs [43–45]. We adopted the same approach in this study. It is reported that an MoE value of  $\geq 10000$  should be considered as “safe,” while an MoE value  $\leq 10000$  could cause a potential risk to public health, and the lower the value, the higher the risk [43, 45]. As shown in Table 2, the MoE value of 126.5 obtained from this study was  $< 10000$ , indicating a significant risk to the consumers of Omena.

Previous studies reported that MoE values for the babies and toddlers were the lowest; this indicates that children might have the highest risk of being exposed to AFB<sub>1</sub> [46, 47]. We have not assessed the risk of AFB<sub>1</sub> exposure for children although the use of pounded Omena as an ingredient in complementary feeding for children is quite common in Kenya. Therefore, studies on the risk of AFB<sub>1</sub> exposure for children are needed to determine the extent of the problem. Also, efforts towards controlling and preventing aflatoxin in the fish value chain should be coordinated and well targeted.

## 4. Conclusion

The demonstrated presence of AFB<sub>1</sub> in Omena at concentrations above the limits acceptable to regulatory bodies is indicative of the risk of the fish as a source of aflatoxin exposure to both humans and animals in Kenya. More often, aflatoxin mitigation measures have targeted major agricultural products, such as cereals, peanuts, and crop by-products. However, it is evident from the results of this study that dried Omena could also be problematic routes for exposure to aflatoxins. Therefore, regular monitoring of dried fish is necessary to understand the extent of the problem.

The present study used Omena consumption data for the whole Kenyan population. Hence, we recommend further exposure assessment studies aimed at providing a comprehensive assessment in other Kenyan cities as the consumption may vary among different cities within Kenya.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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