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Aflatoxin B1 Quantification from Fish Feeds and Fishery Products in Kisumu and Uasin Gishu Counties, Kenya

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Abstract

Mycotoxins are toxic compounds produced by fungi as secondary metabolites. Their level of occurrence has gained global concern, especially in the aquaculture industry, because mycotoxins have shown to be persistent in fish flesh, causing a severe threat to animal welfare and human health. Communities should be aware of the dangers of Aflatoxin so that such knowledge will enable them to handle fish feeds appropriately. This study aimed at establishing contributing factors leading to mycotoxin contamination among farmers and fish feed traders on their effects and possible management strategies. A questionnaire and an observation checklist were used to collect data. A Cross-sectional exploratory study was employed; both qualitative and quantitative data were collected. The Snowball technique was used to identify the respondents. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 24, Binary logistic regression was used to determine associations, and descriptive statistics were used to analyze data. Sixty-two respondents were interviewed; results indicate that more women farmers, 34 (54.8%), participated in the interview than men, 28 (45.2%). Most of the respondents, 53.2%, attained secondary 24.2%, and 11.3% tertiary education in both counties. Among the respondents who have heard of mycotoxins, a quarter (OR: 1.003) of them have heard of Aflatoxins only. Maize 49.7% with a mean of (±14.2) was the primary fish feed ingredient. Out of the 62 samples, 19.4% (±1.33; SD 0.592) were contaminated with AFB1, with the majority of the contaminated feeds 66.7% (±0.25; SD 0.44) were from Kisumu County. Traders were 0.006 times likely to have better knowledge of Aflatoxin than farmers. The findings have shown that education holds a positive and significant correlation with the level of knowledge and occurrence of those who attained secondary (OR 1.078: CI 0.736-1.580) and college (OR 1.050: CI 0.594-1.856). Some of the storage practices were found to influence the exposure of the feeds to the growth of fungi and mycotoxin contamination. Hence, there is a need for more sensitization and training targeting farmers and traders. Additionally, there is a need for a heightened surveillance program to monitor local ingredients, finished feeds, and storage processes.

Keywords: Aquaculture; Aflatoxin; Fish feeds; Mycotoxins; Kisumu; Uasin Gishu

INTRODUCTION

Fish and fish products are an essential source of animal protein for humans, with the most common fish and fishery products consumed in Kenya being Tilapia, *Rastrineobola argentea* (Locally called "*Omena*"), Nile perch (Locally called "Mbuta"), and catfish. Currently, fish consumption rates have gone high, and it is estimated to be up to 450 kg/capita/year due to widely practiced aquaculture, increased population, urbanization, and heightened health benefits (Omasaki, Charo-Karisa & Kosgey, 2013). Mycotoxin contamination has widely been associated with food crops used as ingredients in preparing fish feed, with the most common crops including; Wheat, Barley, Rice, with corn being commonly used because of its availability (Moretti *et al*., 2017). The traditional drying of these cereals is still practiced in Kisumu and Uasin Gishu Counties as a way of preservation; however, they undergo poor handling, processing, packaging, and storage resulting in contamination. Mycotoxin contamination is a public health concern, as these toxins are known to be highly toxic and are mutagenic, teratogenic, and carcinogenic agents (Nakavuma *et al.*, 2020). Mycotoxins are highly potent secondary metabolites of molds that colonize growing crops or harvested and stored animal feeds and food. The most common mycotoxins of importance include; Aflatoxin, Ochratoxin A, Zearalenone, Fumonisin, and Trichothecenes (Afolabi *et al.,* 2020).

Aflatoxins are toxigenic chemicals mainly produced by two major species, *Aspergillus flavus* and *Aspergillus Paraciticus* (Yu *et al.,* 2012). They occur naturally in crops in the form of AF-B1, B2, G1, and G2, with AFB1 being the main contaminant in animal feeds and the most prevalent of all (Oliveira & Vasconcelos, 2020). The toxin is commonly found in areas with hot and humid climatic conditions hence become frequent contaminants of agricultural products and cereals in Kenya. Kisumu county and Uasin Gishu county are prone to aflatoxin because some food grown and consumed are at high risk of AFB1 contamination and prevailing climatic conditions; drought, extreme rainfall, high humidity, and temperatures ranging from 20℃-35℃.

A survey conducted in five counties in Kenya representing different Agro-ecological zones: Isiolo, Kwale, Meru, Bungoma, and Tharaka-Nithi, which was done by (Sirm*a et al.,* 2016), found that aflatoxin levels were high compared to WHO standards of 10 μg/kg in both the animal feeds including fish feeds during the rainy season. Further, the contamination from the feeds was from manufacturing industries which were attributed to poor storage. Research has shown that Aflatoxin has continuously been associated with a high incidence of cancers, especially esophageal and hepatic cancer, in Western Kenya and most parts of the country where contamination is highly frequent.

Cancer is the third leading cause of death in Kenya, and among other cancers, liver cancer has been ranked fourth leading cause of mortality, with hepatocellular carcinoma being identified as the major cause of death (Ochwoto *et al.,* 2019). In addition, the rising incidence rate of cancer in Kenya has remained a public health problem despite the advanced diagnosis and treatment. According to (Mutiga *et al.,* 2015), chronic exposure to Aflatoxin has become a risk factor to increasing disease progression of Human Immunodeficiency Virus and Hepatitis B Virus (HIV and HBV) which are precursors to the development of Hepatocellular Carcinoma (HCC).

On the other hand, there is evidence that the high prevalence of HCC is high in counties with a high prevalence of HIV (Williams *et al.*, 2018). Research has shown that HIV and cancers are the 3rd causes of mortality nationally, with the highest numbers of deaths recorded in Western regions, including Kisumu (Otedo *et al.*, 2018). Reports from other studies show a high prevalence of cancers in Uasin Gishu, with esophageal cancer being the most common. Suggestions from the report showed potential associations from consumption of traditional alcohol (*Busaa or changaa)* attributed to the use of contaminated animal feeds and maize flour used in the fermentation of the alcohol (Kigen *et al.,* 2017). Consumption of contaminated food with Aflatoxin is known to be through dietary exposure that has been evidenced from the past outbreaks (Ezekiel *et al.,* 2018). Direct exposure includes consumption of contaminated food and indirect is through(Voth-Gaeddert, Stoker, Torres & Oerther, 2018) animal products like milk, eggs, and beef which have been studied widely except in fish which have not been studied exhaustively (Sirma *et al.,* 2019).

Contamination of fish has been ignored largely even though high consumption is propagated by increased aquacultural activities and the high production of fish (Marijani *et al*., 2020). Various qualitative and quantitative assessments on Aflatoxin in animal feed and food have been done, with little focus on fish feed which might be the greatest risk factor to cancer development. Due to financial constraints, the Kenyan community practising fish farming have ventured into cheap and contaminated feeds these includes using contaminated ingredients in preparation of fish feed, poor storage and also handling of feeds exerting maximum amount of aflatoxin into the food chain (Njeru *et al.*, 2019).

The few studies that have reported the presence of Aflatoxins in fish feed and exposure is more likely to occur where contaminated fish feed ingredients are used in the preparation, poor methods of fish feed handling and storage that is coupled with the low level of mycotoxin awareness among fish vendors and fish farmers (Tarus *et al.,* 2019). Since mycotoxins, especially Aflatoxin B1is known to be associated with liver cancer, research has shown high consumption due to the high production of fish in Kisumu and UasinNgishu counties (Munyasi *et al.,* 2009). Therefore, this research assessed the level of awareness, fish feed handling, and storage through a baseline survey and determined Aflatoxin levels in fish tissue through laboratory analysis using Enzyme Link Immunosorbent Assay (ELISA).

MATERIALS AND METHODOLOGY

Study Area

The study was conducted in two selected Counties of Nyanza and Rift valley; Kisumu and Uasin Gishu.

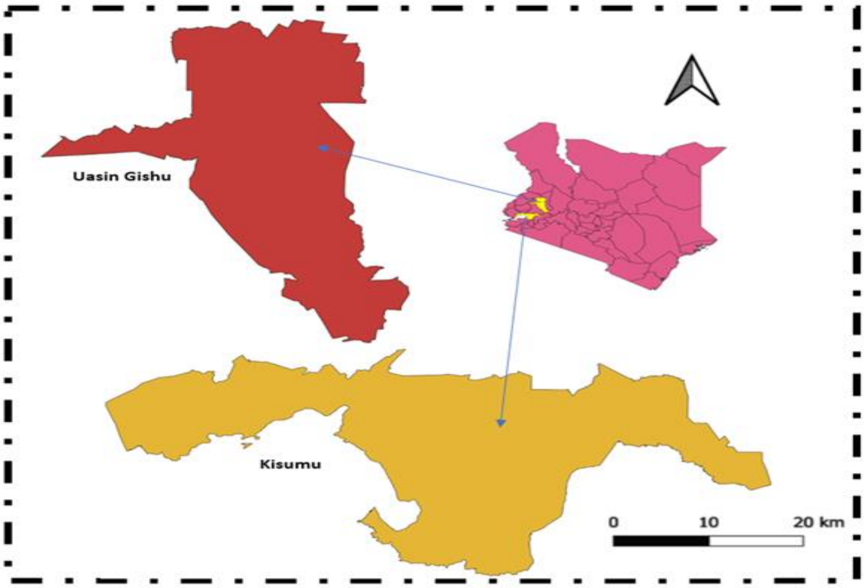

Fig.1 Map of the study area.

Table 1: Agro-ecological characteristics of Kisumu and Uasin Gishu counties where the fish feed samples were collected

County	AEZ	Altitude	Rainfall (mm)	Temperature (˚C)
Kisumu	LM2	1144-1525M	1400-1600 mm	20.9-35.1 **(˚C)**

Uasin Gishu	UM4	1550-1950M	1000-1400 mm	18.0-20.5 (°C)

Abbreviations; AEZ, Agro-ecological zone; LM, lower midland; UM, upper midland
Source; (Njeru *et al.*, 2019).

Sampling Technique, Sample Size, and Data Collection

Fish Farmer's and trader's sample size determination

A field survey was conducted in Kisumu and Uasin Gishu counties. A sample size of 62 was obtained using the coefficient of variation (Nassiuma, 2001). The respondents were recruited using snowballing and purposive sampling methods. A standardized and pre-tested semi-structured questionnaire was used to conduct a face-to-face interview, and an observation checklist was employed in assessing the store's layout and conditions. Proportionate sampling was employed to determine the fish feed sources. The samples were weighed for 10 kg from each sample site then mixed vigorously to achieve homogeneity, then 1kg of the feed sample from each group was selected as have been directed by (Shotwell *et al.,* 1966) to make a composite sample.

Fish feed sampling

The samples were collected from various sources where a list of milling factories, animal feed shops, farmer's stores, and markets were picked purposely. Fish sampling was conducted according to (Nassiuma, 2001) to obtain sampling size. Proportionate sampling was employed to calculate the sample size from each selected site.

Samples collected were weighed, mixed, and sieved using a 20 mm-mesh sieve. Fifty (50) g of the sieved sample were placed in a glass-stoppered Erlenmeyer flask with 25 ml of water, 25 g of diatomaceous earth, and 250 ml of chloroform. The mixture was shaken with a shaker for 30 min. The dough was collected after stirring and filtering to collect 50 ml of the extract. The extracts were then used in the column clean-up.

Column clean-up

A 22- * 300- mm chromatographic tube was used in the clean-up whereby cotton wool was placed in the bottom and 5 g of anhydrous sodium. Chloroform was added till half full, 10 g of silica gel mesh 60 was also added. The column sides were washed with extra chloroform and drained to leave about 7 cm above the silica gel. Carefully 15 g of anhydrous sodium sulfate was added to the top, and chloroform flowed to the top of Na_2SO_4. 50 ml of extract is added from above (Thean *et al*., 1980; Zhang et al., 2020).

Fish tissue sampling tissues

A total of 60 fish were collected from the fish ponds and cages, which were obtained purposively from selected farmers in specific locations. Fish have transported appropriately in a labelled zip, placed in a cool box, transported to Biotechnology Laboratory where they were slaughtered, and approximately 30g of the fish tissues were obtained from each fish. The fish tissues were stored at 15ºC for aflatoxin determination.

ELISA test procedure

A 50 µl of each diluted sample was placed into microwell plates pre-coated with ant aflatoxin antibody. A competitive Enzyme-Linked Immunoassay (ELISA) was performed using RIDASCREEN Aflatoxin total test kit r-biopharma Darmstadt, Germany. The procedure was performed according to the manufacturer's instructions. A sufficient number of microtiter wells were put into the microwell holder to run all the standards and samples. The positions of the standard and samples were recorded. All the results were recorded automatically in a sheet from the aflatoxin reader and interpreted.

Data Analysis

Completeness of entry from the survey data obtained, dual entry, cleaning, and coding were done using Excel Office 13. Analysis was done using SPSS version 21, ANOVA and descriptive statistics was used ANOVA with summarized data presented in tables of frequencies and graphs.

Ethical Consideration

The study was approved by the Human and Animal Research and Ethics Committee (HAREC) of the University of Eastern Africa Baraton, Kenya, and a license to carry out the study was issued by the National Commission of Science Technology and Innovation (NACOSTI), Kenya (NACOSTI/P/19/17980/30579). Formal approval to carry out the study was sought from the Voluntary consent forms to gather for the respect of persons, and confidentiality was assured for any information collected for there were no names written either in the questionnaire, interview schedules, or in the consent sheet.

RESULTS AND DISCUSSION

Survey

Results indicate that 53.2% attained secondary education with 24.2% and 11.3% tertiary education in both counties. Results also indicated that among the respondents, 7 (11.3%) had attained primary education**.**

Table 1: Association between socio-economic traits and knowledge of aflatoxin B1

Social-economic traits		**Knowledge on aflatoxin B1 Kisumu**		**Knowledge on aflatoxin B1 Uasin Gishu**	
		Odds Ratio (95% CI)	**Correlation Coefficient**	**Odds Ratio (95% CI)**	**Correlation Coefficient**
Gender (Male/Female)		0.750 (0.274 – 2.051)	- 0.071	0.750 (0.541 – 1.154)	0.001
Occupation (Trader / Farmer)		1.026 (0.376 – 2.795)	0.006	1.540 (0.85 – 1.787)	0.036
Education	Primary (Yes/No)	0.726(0.168-3.132)	- 0.003	0.369(0.254-2.132)	-0.065
	Secondary (Yes/No)	1.078(0.736-1.580)	-0.067	1.254(0.2547-1.785)	0.005
	College (Yes/No)	1.050(0.594-1.856)	-0.036	1.582(0.248-1.758)	0.065
	University (Yes/No)	0.9(0.261-3.109)	0.036	0.7(0.261-2.108)	0.002
Income (High/Low)		0.357 (0.068 – 1.879)	- 0.251	0.121 (0.015 – 1.578)	0.0251

In regard to that, it was shown in the study that education holds a positive and significant correlation with the level of knowledge and occurrence of AFB1, especially from those who attained secondary (OR 1.078: CI 0.736-1.580) and college (OR 1.050: CI 0.594-1.856), which might have been attributed to the many outbreaks in the recent past and were able to access information from the social media. In Kenya, education has always been considered an important factor for employment in formal and informal setups (Obiero *et al.,* 2019). Research has also shown that educated farmers and vendors can access all key information that can enable them to practice good management practices to reduce mycotoxin contamination (Mutiga *et al.,* 2015; Stasiewicz *et al.*, 2017).

Based on the findings from the study, traders were 0.006 times likely to have better knowledge of aflatoxin than farmers from Kisumu County. This implies a positive association (OR 1.026: CI 0.376-2.795) of the trader's awareness which their occupation might have influenced. This can be due to traders being taken for training on management of mycotoxins through heightened government programs targeting vendors on control of mycotoxins. In comparison with results from the two counties; Both traders and farmers from Uasin Gishu county indicated a significant negative correlation (-0.036) on their awareness of aflatoxin, which the low level of literacy could have attributed. Therefore, packaged information on mycotoxin is key in enhancing the proper management of contamination of fish feeds (Wambui *et al.*, 2016). Although a small proportion of traders were aware of mycotoxin contamination, a significant proportion of the traders, 21 (71%) and 20 (68%) of fish farmers (OR:1.026; CI: 0.376 – 2.795), were not aware of its adverse harmful effects to fish and human health. This concurrence with a study by (Marin *et al.,* 2013). Currently, there is no literature on Aflatoxin's impact among fish feed traders and fish farmers. Therefore, this may indicate poor public awareness (Obade *et al.*, 2015).

Table 2: Type of Fish Feed, Ingredients, and Mode of Preparation

Column 1	***Column2***	**No**	**Mean**	**SD**	**AFB1.No**	**%AFB1**
Feed type	Pellets	35	0.2	0.406	10	34.48%
	Mash	27	0.19	0.396	19	65.52%
Ingredient	Wheat	13	0.23	0.439	3	12.50%
	Maize	34	0.24	0.431	20	83.33%
	Rice	10	0.1	0.316	1	4.17%
	Others	5	0	0	0	0%
Reparation	Home prepared	22	0.18	0.395	12	44.44%
	Factory manufactured	40	0.2	0.405	15	55.56%

In terms of ingredients, most of the respondents, 42 (68%), agreed that maize (49.7% ±14.2) was the primary ingredient in fish feeds, with most of the fish feeds prepared from maize being pellets compared to mash. This could be attributed to maize (*Zea mays, L.*) being the primary and most cultivated cereal crop in Sub Sahara Africa, Kenya, with over 70 million metric tons (Nyagumbo *et al.,* 2018). Maize is the leading staple food in Kenya, contributing to up to 65% as calories (Mutiga *et al.,* 2015). Maize is harvested during the rainy season to avoid the development of mycotoxin when they have matured (Kimanya, De Meulenaer, Van Camp, Baert, & Kolsteren, 2012). Most feed 65.5% and 55.5% sourced from manufacturers were contaminated with Aflatoxins B1 from the results. This could be due to poor handling of feeds during the manufacturing process and storage. As evidenced in Kenya, most of the mycotoxins are detected from maize and maize products, with most detections having been found recently in Makueni, Machakos, Nandi Uasin Gishu, Tran Nzoia, Kisumu and Western (Okoth *et al.,* 2012).

Storage Practices and Disposal of Contaminated Feeds

Figure 2: Cage fish farmer store in Dunga beach, Kisumu.

Figure 3: Farmer's store Uasin Gishu.

Figure 4: Mixed Farmer's Store in Uasin Gishu.

Mixing feeds of all animals kept, e.g., cow, chicken, and fish feeds

On observation, the storage facilities from both farmer's and trader's stores were in poor condition. Improper storage was consistent in most of the stores where fish feeds were stored in unsatisfactory conditions. Therefore, the mean level of aflatoxins detected in the fish feeds can be explained to have been caused by poor storage practices among the fish farmers and traders. An ideal store requires good ventilation that allows air circulation within the store with pallets to support the bags. According to (Munguti *et al.*, 2014), feeds are stored in cool and well-ventilated areas that do not allow exposure to extreme weather conditions like heat and humidity. And as well to be protected from pests that can facilitate contamination. All the stores, both from the farmers and traders, had limited ventilation, with most of them without pellets; however (Queiroz *et al.*, 2012) affirmed that warm environment inside windowless houses and storage of maize on the dirty and unraised floors in feed stores might promote fungal growth.

On conditions that would encourage contamination, respondents from this study were assessed based on their ability to state factors that may prompt the growth of fungi. A good proportion of the farmers, 24 (78.9%), reported high temperature, fish feed traders 22 (70%) mentioned high moisture content, while a few farmers and traders thought that good storage and packaging of fish feeds reduce the growth of molds. In contrast with the observations made, the majority of the farmers packed their feeds in polypropylene bags, as seen in *figure 3*. Polypropylene bags in several studies have been found to contribute to high moisture content, especially in areas experiencing relatively high humidity; this is because they retain some water and does not allow free circulation of air, unlike the sisal sacks, these bags promote the growth mostly when the feed content in the bags have not been dried enough (D'Orazio, 2012). Fungi are aerobic micro-organisms, and recent technologies have been developed that helps in reducing oxygen content and increase carbon dioxide content, therefore, reducing mycotoxin growth (Abigael *et al.*, 2018; Bordin *et al.*, 2015). Currently, in Kenya, hermetic improved bags are being used by heavy commercial traders with few farmers in storing animal feeds. (Rose *et al*., 2018). Other measures reported in other studies included; buying animal feeds in small quantities that can sell quickly (Abbas *et al.*, 2006; Bruns, 2003); unlike what was observed, the most store had piles of stock that have not been sold for more than six months. Hence, exposed to a high risk of contamination.

Figure 5: The Sun Drying of Maize in Uasin Gishu County.

Other probable responses included sun drying to reduce the moisture content; however, contrary observation was made in Uasin Gishu as shown in Figure 4, most of them were drying in an open field on the tarpaulin, which was easily contaminated.

Table 3: The number of feed samples detected with aflatoxin B1

COUNTY	No	MEAN	SD	AFB1. No	% AFB1
Kisumu	32	0.25	0.44	16	0.5714
Uasin Gishu	30	0.13	0.346	12	0.4286

The fish feeds from Kisumu and Uasin Gishu were contaminated 19.4% (±1.33; SD 0.592) with AFB1, with most 66.7% (± 0.25; SD 044) of them from Kisumu County. According to (Mwende *et al.*, 2016), fungi grow at temperatures that range between (10-40℃). However, presence of contamination in Kisumu could be because fungal growth has been shown to be related to extreme weather conditions, high humidity, and relatively high temperature (20.9-35.1° C**)** compared to Uasin Gishu County (18.0-20.5°

C). Findings are consistent with a survey conducted in Western Kenya which falls within the agricultural economic zone with Kisumu and have similar agro-climatic conditions that favor fungal growth (Okoth *et al.*, 2012). These findings, therefore, suggest that Aflatoxin B1 was economically the important mycotoxin contaminant for the fish feeds collected from the region. However, Mutinga lamented that Aflatoxin exposure was widespread across Kenya, especially when no outbreak was recognized, suggesting that contamination is common in fish but has always remained unnoticed due to lack of surveillance, especially in fish feeds. Therefore, there is need for monitoring mycotoxins, even in settings where contamination is not typically high.

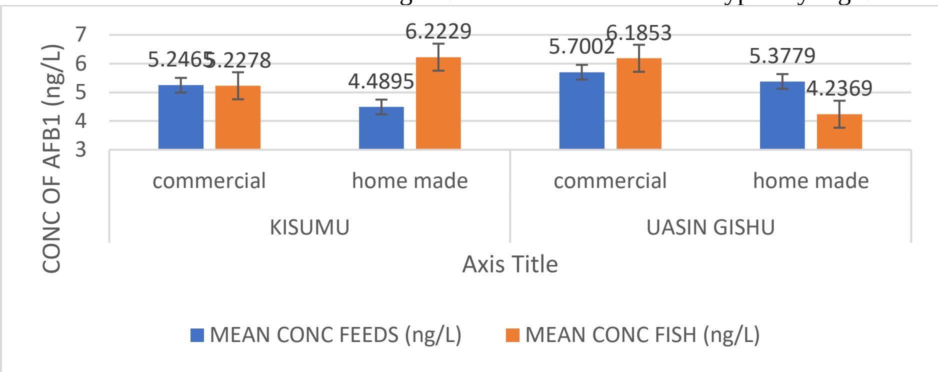

Figure 5: Mean concentration of aflatoxin B1 in fish feeds and fish tissues.

As observed, the mean concentration of Aflatoxin from commercial feeds is higher than feeds produced at home in both counties of Kisumu and Uasin Gishu. This could be due to the length of time the feeds are stored in the trader's stores; most traders have large and poor storage facilities coupled with the hot and humid conditions that encourage the growth of molds. As observed, the mean concentration of aflatoxin from commercial feeds is higher (5.24 ng/L;5.7002 ng/L) than feeds produced at home (4.4895 ng/L;5.3779 ng/L) in both counties of Kisumu and Uasin Gishu.

The total aflatoxin from the fish tissues in Kisumu showed that fish fed with homemade feeds had higher concentrations than those feed with commercial feed, attributed mostly to the type of ingredients used as fish feeds at home. There is a high likelihood that farmers from Kisumu are using *Rastrinieobola argentea* (*Omena)* as their primary ingredient that might have high bioavailability if consumed by fish compared to cereals. Studies have shown that 60%-70% of '*Omena*' in Kisumu are used as human food and fish feeds (Marijani *et al.*, 2020). However, aflatoxin B1 contamination in '*Omena*' due to poor handling, processing, and packaging practices of the commodity is still high. This contrasts with the results from Uasin Gishu; the total concentration from fish tissues was lower in fish fed with homemade feeds. This variation could be due to the large-scale farming of cereals, especially maize, which was their major ingredient in the preparation of fish feed. Several other studies show that a mixture of raw ingredients like what farmers and traders are practicing increases the risk of contamination by fungi to fish feeds (Sirot *et al.*, 2013). Therefore, it is prudent to suggest that strategies to control contamination at both pre and post-harvesting are fundamental to reducing mycotoxins' transfer to feed or food. Therefore, there is a need to understand the amount of Aflatoxin that can bioaccumulate in fish tissues and the possible amount consumed by fish eaters.

CONCLUSION

From the study, there was evidence of contamination of the feeds and fish tissue. Though their low levels of aflatoxin B1 compared to the Kenya standard of 5 ppb, continuous exposure of the toxins through food chain may contribute to health effects to fish and humans. The low levels of knowledge about mycotoxins by both farmers and traders with limited capacity to identify contaminated feeds and pointed out the risk of animal and human consumption were the greatest contributing factors to contamination. Most of the socio-economic characteristics and feed ingredients were significantly associated with contamination of the fish feeds. Some of the storage practices were found to influence the exposure of the feeds to grow molds, suggesting poor practices were employed during packaging and storage. Hence, more sensitization and training are needed to target farmers and traders on the effects of mycotoxin and proper management. Additionally, there is a need for a heightened surveillance program to monitor local ingredients and finished feeds to achieve timely control and reduction of contamination. Public health and other authorities institute routine inspections of storage facilities and capacities to ensure compliance with the set requirements for proper storage.

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