Tropentag 2013
Agricultural development within the rural-urban continuum
University of Hohenheim
Stuttgart- Hohenheim, Germany September 17-19, 2013

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Multi-Mycotoxin Contaminations in Fish Feeds from Different Agro-Ecological Zones in Nigeria

INTRODUCTION

Fish and fish products constitute about 49% of the animal protein consumed in Nigeria with a population of 160,000,000 people (Ajani, 2008). There is high demand for fish as they supply a good nutritional balance of protein, vitamins and minerals with low fat of 10% calories and an excellent source of omega 3 fatty acids**.**

The total fish demand in Nigeria stands at 2.66 million metric tonnes out of which only 0.62 million metric tonnes was produced locally (FDF, 2008), also there is dwindling amount of fish realized from fish capture as a result of overfishing and pollution (Delgado, 2003). The shortfall in the demand is therefore met through importation which is a huge expenses on Nigeria's import bill despite Nigeria's resources for estimated production potential of 2.5 million metric tonnes and that is if only about 20% of the suitable land areas for fish farm are utilized (Omitoyin, 2007; Ajani *et al.,* 2011).

Moulds and mycotoxins contamination reduce feed quality and predispose fishes to incidences of mycotoxicoses when consumed. Fishes are sensitive to mycotoxins causing feeding inefficiencies and poor growth. Pelleted fish feeds when not consumed get soaked and sinks to the bottom thereby polluting the water and causing untimely deaths in fishes resulting to financial losses to fishers, processors and sellers. This aggravates food insecurity in Nigeria.

OBJECTIVE OF THE STUDY

Considering limited information on toxigenic fungi from fish feeds and multimycotoxin contaminations from African countries, particularly in Nigeria, this work will make a contribution to global ecological database of toxigenic moulds and consequent multimycotoxin production from fish feeds in different Agro-Ecological Zones of Nigeria.

MATERIALS AND METHOD

Sampling

Ninety four samples of fish feeds were randomly collected at different locations in the Agro Ecological Zones of Nigeria. Sampling of fish feeds from bags in warehouses and feed mills were done according to the method described by Karthik *et al.,* (2009). The number of bags were sampled using the recommended criteria of FAO (1994). Samples were collected at the top layer, middle and bottom layers. The bulk samples were collected into transparent Zip-lock bags and transported to the Microbiology Laboratory of Nigerian Stored Products Research Institute Ibadan, Nigeria. The samples were communited immediately in order to reduce particle size and stored at -20^{o}C prior to transport to Austria for multi-mycotoxin analysis using LC-MS/MS.

QUANTIFICATION OF MULTI-MYCOTOXINS IN FISH FEED SAMPLES

This was carried out by the method described by Sulyok *et al.,* (2007). The analysis was done at Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna, Konrad Lorenz Str, 20, A-3430 Tulln, Austria. The spectrum of mycotoxins were determined in 94 fish feed samples by LC–MS/MS. Five gram of each homogenized sample was weighed into a 50 ml polypropylene tube (Sarstedt, Germany) and extracted with 20 ml acetonitrile/water/acetic acid (79:20:1, v/v/v) for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany). The extracts were diluted and injected into the instrument as described by Sulyok *et al*. (2007). Screening of the analytes was performed using a QTrap 5500 LC–MS/MS System (Applied Biosystems, CA, US) equipped with a TurboIonSpray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150×4.6 mm i.d., 5 µm particle size, equipped with a C18 4×3 mm i.d. security guard cartridge (Phenomenex, CA, US). The identification of positive analyte was confirmed when two MRMs which yielded 4.0 identification points were obtained according to commission decision 2002/657/EC. In order to distinguish between incomplete extraction and matrix induced suppression of the LC–MS/MS signal, the apparent recovery and the recovery of the extraction step were determined by spiking three individual samples of fish feeds before extraction as well as after extraction on the same concentration level (Sulyok *et al.,* 2006).

STATISTICAL ANALYSIS

Data was analysed using the t-test at 95% confidence level. The t-value, mean differences, standard deviation and standard error of the concentrations of the multi-mycotoxins in fish feed samples were evaluated.

RESULT AND DISCUSSION

The result from table 1 shows that the highest incidence of fish feed contamination was recorded for Fumonisin B_1 with mean of 900.9µg/kg (range of <LOD- 6097.0 µg/kg), followed by Fumonisin B_2 of mean 220.6 µg/kg (range of < LOD- 1203.3 µg/kg) and then Aflatoxin $B_1$of mean 103.0 µg/kg (range <LOD-418.7 µg/kg) while Zearalenone of mean 4.5 µg/kg (range <LOD-26.3 µg/kg) had the least. These values are above the European Union permissible values. The co-occurrence of Fumonisin and Zearalenone in the fish feed samples agrees with the findings of Pietsch *et al.,* (2013) who reported that both mycotoxins were firstly reported in samples of commercial fish feeds sampled from Central Europe. While Hashimoto *et al.* (2003) earlier reported the presence of both aflatoxins and fumonisins in 23.8% of the 42 fish feed samples. These are evidences that fish feeds are carriers of multi-mycotoxins as they are majorly formulated from plant sources that are good substrates for moulds proliferation and subsequent mycotoxins production.

Results from tables 2 and 3 shows Standard deviation, Standard error and Mean difference of the multi-mycotoxin contaminations in fish feed samples from different Agro-Ecological Zones (AEZs) in Nigeria containing Fumonisins, Aflatoxins, Deoxynivalenol, Zearalenone etc whose presence are of concern in the feed industry.

The highest mean value recorded in table 2 was 67.29±7.26 from DON however, highest standard error and standard deviation was from Fumonisin B1. Fumonisin B1 also had the highest toxin value with the range of 0.800-6097µg/kg from Guinea Savannah AEZ of Nigeria. This is in accordance with the observation of Barbosa *et al.,* (2013) who reported that high percentage of the samples (98%) was contaminated with FB1 at high levels. The following mycotoxins were present in all the fish feed samples; Enniatin B, Equisetin, Beauverucin, Emodin, Alternaric methylether, Methyl sterigmatocystin and Averufin toxins while Ochratoxin, T-2 and HT-2 toxins were not detected in any of the sample.

The highly significant effects of the multi-mycotoxin is an indication of variation in geographical and ecological distribution of the toxigenic moulds and the mycotoxins produced. This is in accordance with the findings of Atehnkeng *et al.,*(2008).

In this study, the co-occurrence of multi-mycotoxins of concern in food and feed safety calls for urgent check and policies to standardize the quality of fish feeds utilized in fish farming industry.

Table 1: shows the mean and range level of contamination of fish feeds with some mycotoxins of great importance in food and feed industries from different Agro-Ecological Zones (AEZs) in Nigeria.

Mycotoxins	GS (µg/kg)		HF (µg/kg)		DS (µg/kg)		SZ (µg/kg)		MSZ (µg/kg)		
	mean	range	mean	range	mean	range	mean	range	mean	range	Mean Ave
Aflatoxin B1	119.8	1.1-408.7	90.8	<LOD-418.7	107.4	129.6-150.8	90.4	2.4-319.0	106.8	21.8-255.5	103.0
Aflatoxin B2	14.9	2.8-53.0	11.7	<LOD-69.3	13.8	9.8-35.6	13.8	<LOD-49.0	16.6	2.4-3.0	14.2
Aflatoxin G1	46.0	<LOD-195.8	36.7	<LOD-195.8	41.9	32.9-56.2	34.0	1.3-128.3	38.0	8.7-76.1	39.3
Aflatoxin G2	4.2	<LOD-11.8	5.5	<LOD-11.8	5.3	<LOD-2.4	4.9	<LOD-4.2	4.4	<LOD-2.3	4.9
Aflatoxin M1	5.0	<LOD-17.7	4.2	<LOD-17.7	4.6	3.4-12.1	4.2	1.8-14.9	4.9	1.2-9.0	4.6
Fumonsin B1	1525.4	<LOD-6097.9	723.6	<LOD-4548.3	807.3	794.4-1035.0	787.9	64.9-755.2	660.2	<LOD-1454.5	900.9
Fumonsin B2	377.6	<LOD-1203.3	185.4	<LOD-886.0	202.8	137.2-290.6	180.0	19.7-151.4	157.0	<LOD-281.0	220.6
Fumonsin B3	66.5	<LOD-214.7	56.3	<LOD-210.9	57.6	22.4-38.6	50.7	<LOD-39.7	39.8	<LOD-48.1	54.2
Hydrolysed FB1	11.9	<LOD-53.4	8.1	<LOD-23.0	8.2	6.3-11.2	7.4	<LOD	7.4	<LOD-21.6	8.6
Deoxynivalenol	44.3	<LOD-234.7	102.5	<LOD-334.0	97.3	5.4-86.5	92.5	11.7-176.8	92.7	<LOD-261.9	85.9
DON-3-Glucoside	2.9	<LOD-10.2	5.6	<LOD-27.4	5.4	2.34-5.6	5.2	0.6-8.7	5.3	<LOD-11.6	4.9
Zearalenone	5.1	0.2-22.1	4.8	<LOD-26.3	4.5	0.3-6.0	4.1	0.5-7.0	4.0	0.2-7.9	4.5
Nivalenol	15.1	<LOD-65.2	8.0	<LOD-14.0	7.5	<LOD-4.7	6.2	<LOD-5.5	6.0	<LOD-7.9	8.5
Average	172.2		95.6		104.9		98.6		87.9		

KEYS: DS- Derived Savannah, HF- Humid Forest, GS-Guinea Savannah, SZ-Savannah Zone and MSZ- Mangrove Savannah Zone.

Table 2 shows Mean and Standard deviation of the concentration of the multi-mycotoxins of food importance in the fish feed samples

MYCOTOXINS	MEAN ± STD ERROR	STANDARD DEVIATION
Aflatoxin B_1	1.03 ± 11.80	116.22
Aflatoxin B_2	12.52 ±1.49	14.72
Aflatoxin G_1	35.99±4.74	8.71
Aflatoxin M_1	2.80±0.88	8.02
Fumonisin B_1	8.59±124.37	1224.90
Fumonisin B_2	2.00±33.46	329.51
Fumonisin B_3	34.00±5.21	51.31
HFB_1	5.56±1.00	9.74
DON	67.29±7.26	71.53
DON3GluCOSIDE	4.63±1.02	10.07
Zearalenone	5.58±1.15	11.31
Nivalenol	5.33±1.20	11.81

Table 3 shows T-test and mean difference of the level of multi-mycotoxin concentration in the fish feed samples.

MYCOTOXINS	T-VALUE	MEAN DIFFERENCE
Aflatoxin B_1	8.76**	103.38
Aflatoxin B_2	8.38**	12.52
Aflatoxin G_1	7.60**	35.99
Aflatoxin M_1	5.55**	4.52
Fumonisin B_1	6.91**	858.89
Fumonisin B_2	5.96**	199.22
Fumonisin B_3	6.52**	33.97
HFB_1	5.62**	5.56
DON	9.26**	67.29
DON3GluCOSIDE	4.52**	4.63
Zearalenone	4.86**	5.58
Nivalenol	4.45**	5.33

ACKNOWLEDGMENT

Special thanks to Almighty God for the successful completion of this work

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