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A cross-sectional evaluation of aflatoxin B1 and M1 contaminations of dairy cattle production in Northern Nigeria

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In Nigeria, dairy industry holds monumental prospects in the management of protein deficiencies among the timid Nigerian populace. Emergence of metabolic products of some important fungi, Aflatoxins B1 and M1 (AFB1 and AFM1), may hamper such potentials and poses public health threat to the consumers of dairy products. Hence, the need to undertake a study with the view of evaluating AFB1 and AFM1 levels in dairy cattle production. A total of 180 samples, each of cattle feed and cow milk were analyzed using Cobra cell incorporated High Performance Liquid Chromatography (HPLC) technique. Significant number of feed (89%) and milk (94%) turned out positive for AFB1 and AFM1, respectively. Factors of production such as the holding-capacity (size) of the dairy herds, type of dairy herds and the type of dairy cattle feed were used to evaluate and determine the occurrence of the toxins. Results showed that most of these factors affect the distribution of the toxins significantly ($P<0.05$). Traditional dairy herds, which constitute the greatest part of the small-holder dairy herds, showed the largest significant number of farms ($P<0.05$) with detectable levels of AFB1 and AFM1 above the acceptable concentration limits in fresh cow milk. It is recommended that critical factors of dairy production be given thorough regulatory considerations as they were observed to play significant role in the occurrence of aflatoxins in dairy products. Also, the management of the traditional dairy herds should be properly guided by the relevant legislation as it constitutes greater part of the dairy production in Nigeria.

Key words: Dairy production, Aflatoxin B1, Aflatoxin M1, feed, cow milk, Northern Nigeria.

INTRODUCTION

Aflatoxins (AFs), are toxic secondary metabolites of some important species of *Aspergillus* particularly *Aspergillus flavus* and *Aspergillus parasiticus*. These fungal species remain one of the important naturally occurring

contaminants of both feed and food ingredients (Hedayati et al., 2007). The organisms tend to elaborate their toxic metabolites under favorable climatic conditions of temperature, humidity and moisture (Tvrtkovic, 2006).

Other factors which increase the optimal liberation of toxins by these organisms include poor storage condition and stress due to pests (Naidoo et al., 2002).

The knowledge of the toxins came to be in the early 1960s when an incidence of mortality struck a total of 100,000 turkey poultries in the United Kingdom. The deaths of the turkeys were investigatively attributed to acute liver necrosis and bile duct hyperplasia after they consumed contaminated groundnuts (Asao et al., 1965). Aflatoxin-producing strains of *A. flavus* and *A. parasiticus* produce four major classes of aflatoxins. These are AFB1, AFB2, AFG1 and AFG2 in addition to major metabolite derivatives such as aflatoxin M1 (AFM1). The metabolites are usually produced either by host body metabolism (humans, animals and microorganism) or by environmental reactions (Williams et al., 2004). AFB1 was found to be the most common and toxigenic of all foodborne mycotoxins found in human foods and animal feeds (Hedayati et al., 2007).

Aflatoxins belong to group 1 carcinogens under which AFB1 is categorized as a definite carcinogen (that is, class 1 carcinogen) for humans (IACR, 1993). It is also worthy of note that aflatoxin M1 (AFM1), the principal hydroxylated metabolite of AFB1, was once classified as group 2B carcinogen to humans by the International Agency for Research on Cancer (IARC, 1993; Kang'ethe and Lang'a, 2009). However, such classification was recently considered erroneous as further investigation demonstrated its *in vivo* genotoxicity and cytotoxicity effects (Caloni et al., 2006). Re-classification of AFM1 has been considered to belong to group 1 human carcinogen (IARC, 2002). Excretion of AFM1 in milk by lactating animals takes place within 12 h of ingestion of contaminated feeds with AFB1 (Battacone et al., 2003). The amount of AFM1 recovered from ingested AFB1 is variable and is mostly dependent on the concentration of the ingested AFB1 and the animal and it ranges between 1-3% (Fremy et al., 1988). Aflatoxin B1 in the range of 0.3-6.2% has been estimated to be transformed to AFM1 in milk (Creppy, 2002). Aflatoxin M1 has been shown to be hepatocarcinogenic at 50 ppb in Fischer male rats with potency of 2-10% of the parent compound and also to induce low incidences of intestinal adenocarcinomas (Cullen et al., 1987).

According to the US FDA, action levels for AFB1 and AFM1 of 5 and 0.5 ppb respectively in dairy products, the potency of AFM1 is swiftly believed to represent one tenth of the parent compound, AFB1. In spite of this assumed gap, AFM1 still represents a potential carcinogen for humans (Williams et al., 2004). The contamination of dairy products with AFM1 is considered

a significant human health hazard, particularly for babies who depend wholly on milk for survival. The tolerance limit for AFM1 in milk varies from one country to the other. The European Union (EU) and Codex Alimentarius Commission put an action level for AFM1 in liquid milk at 50 ng/L whereas the FDA of the USA operates a more relaxed safety level of 500 ng/L (Codex Alimentarius Commission, 2001).

In Nigeria, reported prevalence rates of AFB1, strongly indicated that both humans and animals are highly exposed (Ekhuemelo and Abu, 2018; Akinmusire et al., 2019; Ezekiel et al., 2019). In the northern part of the country, where significant proportion of dairy cattle population is recorded, high level of exposure to AFB1 had also been reported (Omeiza et al., 2018). Animals in this part of the country depend largely on preserved feed and supplements for survival due majorly to low rain fall; and in situations where the feed does not meet the required storage conditions, there is high possibility of contamination of the feed by AFB1 which is easily metabolized into AFM1 and excreted through milk. Most previous works concentrated on AFB1 and not much had been reported with regard to AFM1 which directly affect human health through the consumption of milk. Aflatoxin B1 contamination of feed above the allowable limits may result in the corresponding level of AFM1 in milk. This perhaps, presents challenges to the vulnerable local pastoralist, who, on most occasions, consumes fresh milk directly from the udder of cows. Also, vulnerable young children are mostly weaned to depend on cow milk and milk products as their primary sources of protein.

Children in their early age and many chronically ill individuals are known to possess incompetent immunity (Mwanza, 2011), therefore, consumption of cow milk contaminated with AFM1, may further suppress their vulnerable immunity, thereby increasing their susceptibility to diseases. This study was designed, therefore, to evaluate aflatoxins B1 and M1 contamination of dairy cattle production particularly in the Northern part of Nigeria, where more than 60% of dairy cattle population settle.

MATERIALS AND METHODS

Sampling structure

Dairy cows of producing age were the target population. At the time of this study, active local dairy herds, commercial and institutional dairy farms, constituted the sampling frame. Farms and herds intended for sampling were strictly selected based on the then current dairy activity and profile as supported by the available

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production records. Local herd groups were also selected based on the observed boosting and complementary capacity. Feed and milk samples were therefore concurrently collected from selected farms and/or herds and local dairy herd groups attached to either commercial or institutional dairy farms where local dairy products were purchased as means of boosting milk production capacity of the conventional farms.

Milk sampling

A cross-sectional study was conducted in six dairy farms comprising of commercial and institutional dairy farms. In addition, three Fulani herd groups were also included in the sampling; giving room for inclusive studies for both conventional and traditional dairy settings as commonly practised in Nigeria. From each farm, two fresh milk samples of about 40 ml each were randomly collected weekly for a period of 10 weeks. In the end, a total of 180 samples were collected for study.

Feed sampling

The same sampling pattern as applied under milk sampling was adopted here. Sample sources were the selected commercial farms, institutional farms and Fulani herd groups. Feed samples were collected from the above mentioned farm types as fresh and preserved where applicable. Sterile polytene bags and iron probes were purchased and used for sample collections from both feeding troughs and preserved feed in stocks respectively. In the case of preserved or stored feed samples, a modified systematic sampling technique was adopted. An imaginary diagonal line was drawn across the stored bags of feeds and bags were selected along the line with already defined regular intervals maintained between them. These selected bags were probed each at different points to pool an estimated representative sample of averagely 40 g. Simple random sampling technique was at this point used to directly collect fresh feed samples from the troughs. One representative sample was collected in each case and at every visit until a total pooled feed samples reached 180.

Extraction and Purification of Aflatoxins AFM1 and AFB1

Extraction of milk sample for AFM1

Twenty milliliters of a fresh milk sample (full-cream milk) was pipetted into a test tube and incubated for 30 min at 4°C. It was then subjected to centrifugation at 3000 g for 10 min. A total of 1800 µl of the clear milk serum below the fat layer was taken off and mixed with 200 µl methanol. This solution was collected and kept at -20°C in amber colored vials for further analysis.

Extraction of feed sample for AFB1

A 20 g particle size of each of the feed sample collected was prepared for extraction. An extraction solvent of 80% strength was prepared by adding 20 ml of distilled water to 80 ml of acetonitrile for each sample to be extracted. The prepared extraction solvent in the quantity of 100 ml was transferred to a container. The 20 g feed sample was ground and then added, bringing the ratio of sample : solvent to 1:5 (w/v). The resulting mixture was blended and homogenized using Stomacher blender® for a minimum of 2 min. The mixture was allowed to settle and the extract filtered through Whatman No. 1 filter paper. The filtrate was collected in amber vials

for ELISA or any other analysis.

AFM1 and AFB1 Clean-up procedure using Immuno-affinity column (IAC) technique

A 5 ml aliquot of filtrate of the feed and a 2 ml filtrate of the milk samples were individually and separately diluted with 14 ml of phosphate buffered saline (1xPBS) solution (8.0 g NaCl, 1.2 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, dissolved in 990 ml purified water) and pH adjusted to 7.0 with HCl. The diluted filtrates of feed and milk samples were separately passed through the Aflatest® IAC at a flow rate of 2 ml per minute to enable the aflatoxin to be captured by the antibodies present in the column. After that, the column was washed with 20 ml of 1xPBS at a flow rate of 5 ml per minute in order to remove the unbound material, until air passed through the column. Aflatoxins were released from the column following elution with 1 ml of 100% methanol at a flow rate of 1 drop per second and 1 ml of water passed through the column and collected in the same vial to give a total of 2 ml. The eluate (AFs extract) was collected in amber vials, evaporated to dryness using stream of nitrogen gas at 50°C and stored at +4°C.

Quantitative determination of AFB1 and AFM1 using high performance liquid chromatography (HPLC)

Shimadzu Prominence UFLC Liquid chromatography system (Kyoto, Japan) was used for the HPLC determination. It consists of a Liquid Chromatography, LC-20AD which is fitted to a degasser, DGU 20A_{5R}, auto sampler (injection) SIL 20A, communication bus module CBM 20A, column oven CTO 20A, photodiode array detector SPD M20A and fluorescent detector RF 20A XL, connected to a gigabyte computer with Intel Core DUO and Microsoft XP operating system. The analytes that fluorescence was detected at specific excitation and emission wavelengths also referred to as the compound's fluorescence signature. Extracts from IAC were dissolved in 500 µl of HPLC grade acetonitrile. Samples were run at a flow-rate of 1 ml per minute (min⁻¹) retention times. Aflatoxin analysis involves the coupling to the detector a coring cell (CoBrA cell) (Dr Weber Consulting, Germany) as an electrochemical cell for the derivatization of aflatoxins. The following mobile phases were used for the analysis of Aflatoxins- Methanol/Acetonitrile/Water (20/20/60, v/v/v) containing 119 mg of potassium bromide (KBr) and 350 µl of nitric acid (4M HNO₃).

Recovery analysis

In order to confirm the efficiencies of the extraction procedure, recovery analyses were carried out. Triplicate spiked samples of feed and milk in concentrations of 5, 10 and 20 µg/g of AFB1 standards and 75, 150 and 300 ng/g of AFM1 standards were prepared. The prepared standards were extracted using the same techniques described above. The resulting extracts were purified and analyzed using Immuno Affinity columns and HPLC. Efficiency of extraction was measured in terms of percent recovery which was determined as the average recovery rate.

Statistical analysis

Data obtained from this study were organized and analyzed using Graphpad prism version 5 statistical software. One way ANOVA statistical package was employed to analyze the grouped data. Means were compared using Newman-Keuls Multiple Comparison Test as post-hoc. Statistically significant values were fixed at p

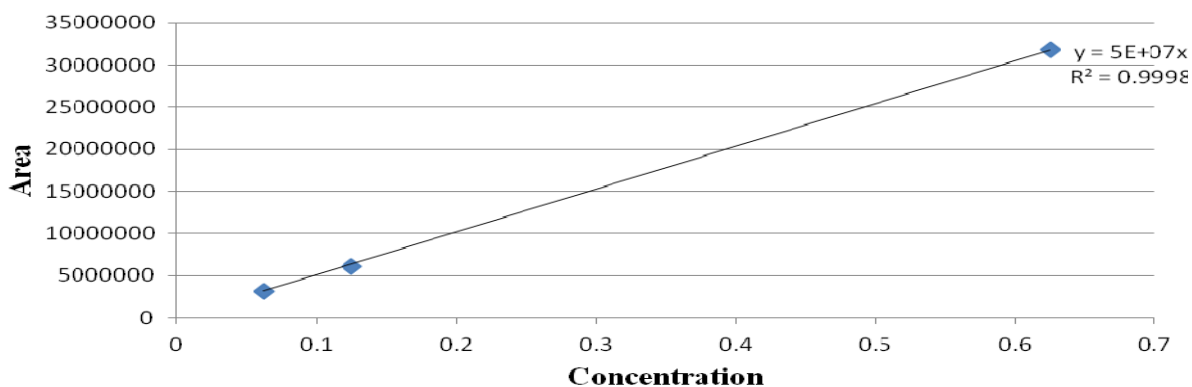


Figure 1. HPLC based standard calibration curve for AFM1.

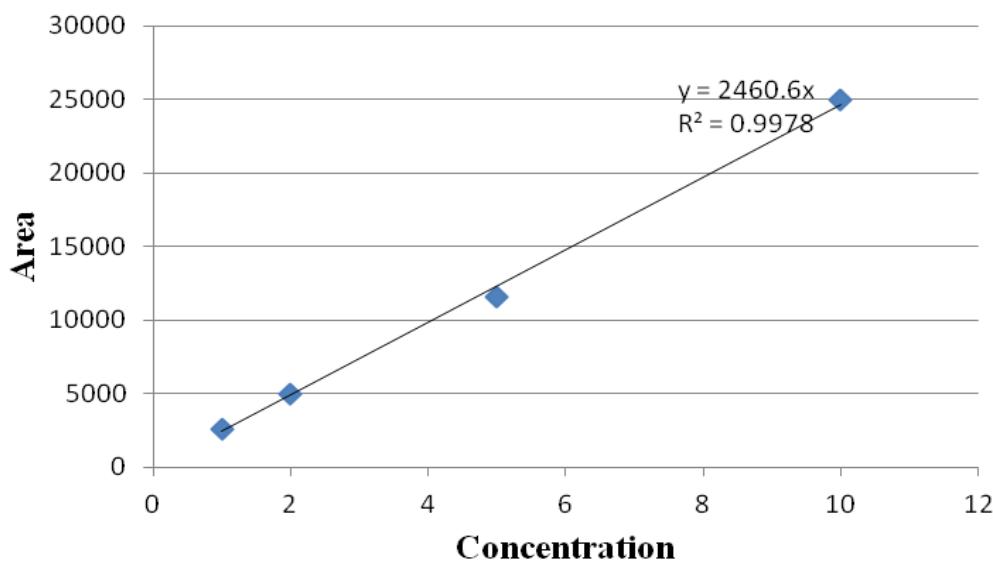


Figure 2. HPLC based standard calibration curve for AFB1.

value less than 0.05.

RESULTS

Occurrence of AFM1 in fresh cow milk and AFB1 in dairy cattle feed

Concentration levels of both AFM1 and AFB1 were determined using prepared standard calibration curves as shown in Figures 1 and 2). A total of 170 (94%) and 160 (89%) of the tested samples turned positive for AFM1 and AFB1 respectively. Of the AFB1-positive samples, 157 (98.1%) showed levels of contamination equating or exceeding 5 µgKg⁻¹, out of 35 (21.9%) which had AFB1 contamination levels of up to and above 20 µgKg⁻¹ (Table

1). Also, 46 (27.1%) out of the 170 AFM1 positive milk samples showed levels of contamination equating or exceeding 0.5 µgL⁻¹ (Table 1).

Distribution of AFB1 concentrations in relation to feed types

Aflatoxin B1 (AFB1) was analyzed according to the different feed composition commonly used to feed dairy cattle in Nigeria. The distribution of the AFB1 according to the different feed composition and formulation is presented in Table 2. There was no statistically significant difference (p>0.05) between the feed of concentrate origin and feed of pure grains. However, both feeds of concentrate origin and grains showed significant

Table 1. Occurrence of AFB1 and AFM1 among the different dairy herd institutional management systems.

Sampled dairy herd	Type of dairy herd institutional management system			No. of collected samples		No. of positive samples (%)	
	Institutional	Commercial	Traditional	Dairy cattle feed	Fresh cow milk	AFB1 in feed	AFM1 in milk
NP	*			20	20	17(85)	19(95)
DC	*			20	20	19(95)	20(100)
YS		*		20	20	15(75)	18(90)
CG	*			20	20	20(100)	20(100)
JM		*		20	20	13(65)	15(75)
GG		*		20	20	16(80)	18(90)
TH			***	60	60	60(100)	60(100)
Sub-total	60	60	60	-	-	-	-
Total		180		180	180	160(89)	170(94)

* indicates the type of dairy institution and the number of herds in that institution e.g.

*** indicate the institution comprises of 3 dairy herds sampled for analysis.

Table 2. Determination and distribution of AFB1 based on the type of dairy cattle feed.

Type of dairy feed	No. of samples collected and analyzed	No. of positive samples	Determined mean concentration \pm SD (μgKg^{-1})	No. of positive samples at critical levels of concentrations of AFM1 (μgKg^{-1})				
				< 5.0	5.0-9.99	10.0-14.99	15.0-19.99	\geq 20.0
Feed + concentrates	60	54	21.7 \pm 7.9	0	0	28	8	18
Grain only	60	60	18.4 \pm 9.2	0	7	15	21	17
Forage only	60	46	10.8 \pm 6.2	3	17	25	1	0
Total	180	160	-	3	24	68	30	35

differences ($p < 0.05$) when compared with the feed formulation comprising of purely pasture, in relation to AFB1 level of contaminations and distribution (Table 2). Feed formulation comprising of concentrates, followed by feeds of grain origin showed higher proportions of positive feed samples at concentration range of $\geq 20 \mu\text{gKg}^{-1}$ (Table 2).

Effects of farm holding-capacity on the occurrence of AFM1 and AFB1

Data collected from these groups of dairy farms were subjected to statistical analysis and results presented in (Tables 3 and 5). There were no statistically significant differences ($p > 0.05$) between the different sizes of dairy farms in relation to the determined AFB1 mean concentrations (Table 3). However, traditional Fulani dairy herd groups constituting largely the small scale dairy farms, significantly ($p < 0.05$) showed the highest number of positive feed samples with detectable AFB1

concentration of $\geq 20 \mu\text{gKg}^{-1}$ (Table 3). Conversely, the different sizes of the dairy farms showed a statistically significant difference ($p < 0.05$) with regards to the determined mean AFM1 concentrations and the milk samples which turned positive for AFM1 at critical concentration of $\geq 0.5 \mu\text{gL}^{-1}$ (Table 5).

Small scale dairy farms of cattle population ≤ 50 showed the highest number 34 (21.3%) of positive samples which were contaminated with AFB1 at concentration range of between 10 and $\geq 20 \mu\text{gKg}^{-1}$. Large and medium farms having dairy cattle populations (≥ 150) and (51-149) respectively showed lower levels of AFB1 contaminations within the same concentration ranges (Table 3).

Comparative distribution of AFB1 and AFM1 positive samples amongst the various types of dairy herd institutions

Table 4 displays the levels of AFB1 contaminations

Table 3. Determination and distribution of AFB1 based on the holding-capacity of dairy herd institution.

Dairy herd type	No. of samples collected and analyzed	No. of positive samples	Determined mean concentration \pm SD (μgKg^{-1})	No. of positive samples at critical concentration of AFB1 (μgKg^{-1})				
				< 5.0	5.0-9.99	10.0-14.99	15.0-19.99	\geq 20.0
Large (\geq 150 cattle population)	60	56	15.2 \pm 10.6	3	24	29	3	2
Medium (51-149 cattle population)	60	44	19.9 \pm 7.1	0	0	26	7	11
Small (\leq 50 cattle population)	60	60	21.4 \pm 8.2	0	0	18	20	22
Total	180	160	-	3	24	68	30	35

Table 4. Determination and distribution of AFB1 based on the type of dairy herd systems.

Dairy herd type	No. of samples collected and analyzed	No. of positive samples	Determined mean concentration \pm SD (μgKg^{-1})	No. of positive samples at critical concentration of AFB1 (μgKg^{-1})				
				< 5.0	5.0-9.99	10.0-14.99	15.0- 19.99	\geq 20.0
Institutional	60	56	10.2 \pm 5.6	3	20	25	8	0
Commercial	60	44	16.7 \pm 10.2	0	4	20	10	10
Traditional Fulani	60	60	19.4 \pm 7.2	0	0	23	12	25
Total	180	160	-	3	24	68	30	35

Table 5. Determination and distribution of AFM1 based on the holding-capacity of the dairy herd institution.

Dairy herd holding-capacity	No. of samples collected and analyzed	No. of positive samples	Determined mean concentration \pm SD (μgL^{-1})	No. of positive samples at critical levels of concentrations of AFM1 (μgL^{-1})			
				< 0.050	0.050-0.099	0.100-0.499	\geq 0.50
Large (\geq 150 cattle population)	60	54	0.12 \pm 0.03	0	23	31	0
Medium (51-149 cattle population)	60	56	0.32 \pm 0.21	0	0	42	14
Small (\leq 50 cattle population)	60	60	0.38 \pm 0.31	0	9	19	32
Total	180	170	-	0	32	92	46

according to the different types of farm (that is, institutional, commercial and traditional Fulani herd groups). There was no statistically significant difference ($p > 0.05$) in AFB1 mean concentrations among the different types of dairy farms. But, the determined AFB1 mean concentration in Fulani dairy herd groups was higher than either of the commercial or institutional farm which had comparatively lower level of AFB1 concentration. However, there was statistically significant difference ($p < 0.05$) between the traditional Fulani dairy establishments with regard to AFB1 positive feed samples at critical concentrations in the range of 10 and $\geq 20 \mu\text{gKg}^{-1}$ and any of the commercial and institutional farms (Table 5). It was observed that institutional farms did not show any positive samples with regards to either feed or milk samples at AFB1 and AFM1 critical concentrations of $\geq 20 \mu\text{gKg}^{-1}$ and $0.5 \mu\text{gL}^{-1}$

respectively (Tables 4 and 6). Various types of dairy farms involving Institutional, Commercial and Fulani dairy herd groups were studied for comparative AFM1 evaluations. Mean AFM1 concentrations \pm SD for the different farm types were determined as presented in (Table 6). The different dairy farm types showed statistically significant differences ($p < 0.05$) in relation to their determined AFM1 mean concentrations. It was observed that, Fulani dairy herd groups showed the highest mean AFM1 concentration \pm SD when compared with either commercial or institutional farm types (Table 6).

DISCUSSION

Mycototoxins which are produced largely by fungi have

Table 6. Determination and distribution of AFM1 based on the type of dairy herd institution.

Dairy herd type	No. of samples collected and analyzed	No. of positive samples	Determined mean concentration \pm SD (μgL^{-1})	No. of positive samples at critical concentration of AFM1 (μgL^{-1})			
				< 0.050	0.050-0.099	0.100-0.499	\geq 0.50
Institutional	60	59	0.22 \pm 0.13	0	32	32	0
Commercial	60	51	0.33 \pm 0.21	0	0	30	20
Traditional Fulani	60	60	0.44 \pm 0.25	0	0	30	26
Total	180	170	-	0	32	92	46

accounted for high economic losses through reduced animal production, trade barriers for consumable food items and direct loss of lives (Wu, 2006) reported by Udom et al. (2012). Increasing growth in the incidences of *A. flavus* aflatoxins has shared a large proportion of the health concerns due to its high carcinogenic profile documented across the globe (Trucksessy et al., 2002; Vaamonde et al., 2003; Melki et al., 2007), Africa (Strosnider et al., 2006) and Nigeria (Okonkwo and Obionu, 1981; Bankole et al., 2004; Omeiza et al., 2018). In particular AFB1, a known second largest cause of liver cancer, when ingested by ruminants, about 1-2% of it is converted to its metabolite, AFM1 which is majorly excreted in milk (Fink-Gremmels, 2008). AFM1 has been incriminated to have as high carcinogenic potency as the parent compound (AFB1) (Henry et al., 2001). In effect, it thus implies that the synergistic effect of both AFB1 and AFM1 may bear worst economic implications on animal health and production on one hand and human health on the other hand. Few amongst other health implications reported include carcinogenic, teratogenic, hepatogenic, mutagenic and immunosuppressive effects in animals and man. These clinical effects coupled with anemia, a prominent clinical feature of aflatoxicosis in dairy cattle, have led to severe milk drop due to reduced feed intake by the affected animals (Akanke et al., 2006).

In the current study, feed and milk samples showed varied monolithic proportions of detectable levels of AFB1 and AFM1, respectively. This variation, perhaps, alluded to the high sensitivity and specificity associated with the coring cell (CoBra cell) detector used as an electrochemical tool for the derivatization of aflatoxins (Mwanza 2011). Significant proportions of the feed samples in the range of $\geq 80\%$ showed considerable detectable levels of AFB1 between (5 and ≥ 20 ppb). This aspect of the finding also agrees with the report of Udom et al. (2012) who separately reported an incidence of as high as 91.7% across dairy samples tested for aflatoxins in a different region of the country. In comparative terms, the AFB1 concentrations, both in the current and previous findings, have demonstrated high level of contravention exceeding the AFB1 concentration limits of 5-10 ppb and 10-20 ppb set by the European Union (EU)

and FDA respectively for dairy industries.

One very important finding of this study was the higher AFB1 concentrations of between 90 and 100% prevalence rates found in the premix-formulated feed compared with the other feed formulations of ruminants. Conversely, it is only a 75% proportion of the tested dry pasture, majorly grasses that showed positive results for AFB1. This could be due to the presence of supplements (concentrates) in the feed formulation. Accensi et al. (2004) had demonstrated the common distribution of AFB1 in cereal plants. According to his report, AFB1 in leaves and stalk showed lower concentrations when compared with cobs and grains. This may suggest the possible reason why the grass samples in this study showed a relatively low level of AFB1 concentrations when matched with the other dairy feed formulations.

Distribution of AFB1 levels based on the type and holding-capacity of the sampled dairy herds did not show any significant differences ($P > 0.05$). However, comparative evaluation of the mean concentrations among the variables, showed considerable differences ($P < 0.05$) between them. Small scale dairy herds (consisting majorly of traditional herd groups and few private farms) showed higher levels of AFB1 mean concentrations. This could be due to poor husbandry management in terms of processing and storage of feeds (Al-Delamyi and Mamoud, 2015). In some instances, however, comparative absolute levels of determined AFM1 showed no significant difference between (institutional, commercial) and local dairy herd groups. Many of the small scaled institutional and commercial farms were observed to be treading the same pattern of husbandry management with the traditional dairy herds. Also, some of the farms with large-holding capacity were observed to be highly dependent on the dairy produce from the local dairy herds. This may be unconnected to the insignificant difference noticed in the levels of AFM1 concentrations between the 'large scale' and the 'small scale' dairy farms.

Aflatoxin M1 (AFM1) is a metabolic product of AFB1, which finds its way in to the mammary excretion (milk). The FDA limits for both AFB1 and AFM1, are 5 ppb (in dairy feed) (Udom et al., 2012) and $0.5 \mu\text{gL}^{-1}$ in dairy products (Diaz et al., 2004). By implication, the acceptable

limit of AFM1 is apparently 10-fold lower than the tolerance level of the parent compound, AFB1, in dairy feed. In spite of this comparatively lower concentration of AFM1, its potency in terms of carcinogenicity in humans is largely compared with the AFB1 (Henry et al., 2001). This stresses the health risk exposed to the consumers of traditionally processed dairy products in this part of the world, where monitoring is minimal.

In the current study also, significant proportion (> 90.0%) of dairy samples collected for AFM1 analysis turned positive at critical levels of detection. This raised health related concerns amongst the health workers who perhaps have the glimpse of the health risks associated with its consumption. It became more worrisome to see that from the data analyzed, about 27% of the analyzed dairy products had detectable levels of AFM1 at $0.5 \mu\text{gL}^{-1}$. Udom et al. (2012) clarified on the kinetics surrounding the biotransformation of AFB1 in dairy cattle. Ingested AFB1 in cattle undergoes biotransformation in the liver and excreted into milk within 12 h. AFM1 levels as low as $0.5 \mu\text{gL}^{-1}$ in milk is considered unsafe as against $5.0 \mu\text{gKg}^{-1}$ which is considered unsafe with respect to AFB1 in dairy cattle feed. This also explains the risk associated with the direct consumption of beef from animals that fails to follow routines abattoir practices.

This study has shown that small scale dairy producers have demonstrated significant level of contravention. Of this group, traditional Fulani herds showed the highest level of contravention of the globally acceptable limits of aflatoxins in both feed and dairy products. Small scale dairy production, which holds more than 60% of the total dairy output in Nigeria, is observed in this study to be more prone to sub-standard farm practices with the attendant consequences of poor hygiene and handling of dairy products (Al-Delaimy and Mamoud, 2015). Fulani dairy products (fresh and processed) have constantly and significantly impacted the economy and health of the nation. Dairy products from pockets of small scale dairy producers are routinely purchased by well established large farms to boost their production and supplies. It is also a known fact that considerable proportions of people comprising majorly of low economic status and partly middle class status in Nigeria depend largely on this sector for protein supplementation. It is therefore a great public health concern, that, more than 25% of the analyzed cow milk showed detectable levels of AFM1 at concentrations $\geq 0.5 \mu\text{gL}^{-1}$ in this study. This poses tremendous health risk to the larger population that consumes these products. Some small scale and medium scale institutional farms were found to produce dairy products which contain detectable level of AFM1 in lower proportions of 18.2% and 8.2% respectively among other farms sampled in this study. Much lower and highly insignificant proportion of (0%) detectable levels of AFM1 at concentrations $\geq 0.5 \mu\text{gL}^{-1}$ was observed among the large and well established institutional and commercial

farms.

The higher and moderate levels of contravention seen in the case of small and medium dairy establishments, when compared with the insignificant contravention level associated with the large dairy herds, may not be unconnected to poor hygiene and handling and non-compliance to standard husbandry practices. In addition, desperate attempts in making high economic returns which undermines health, amongst producers of dairy products and the loose legislation guiding the dairy industries in Nigeria may be implicated also. This strongly highlights the imperative of the need to promulgate laws which incorporate all components of dairy production in Nigeria, inclusively and importantly the traditional Fulani dairy herds.

Conclusion

This study showed that significant proportions of dairy feed and cow milk, in this part of the world, are highly contaminated by AFB1 and AFM1 respectively. Critical factors of production have been identified to affect the level of Aflatoxin contaminations of dairy production in Nigeria. These critical factors of production, if considered and built in the national control programmes and policies guiding dairy production, may significantly reduce exposure risks to the lethal effects of aflatoxins AFB1 and AFM1 to the consumers of cow milk and their products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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