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# Quantitative Analysis of Aflatoxin $M_1$ in Samples of Branded and Unbranded Cow Milk in Kaduna Metropolis

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**Abstract:** *Quantitative determination of Aflatoxin M1 was investigated in this study. Twenty four (24) samples of unbranded cow milk (UCM) and fifteen (15) various brands of branded cow milk was used in this study. ELISA kits were used to measure Aflatoxin M1 present. The levels of AFM1 obtained in the milk were significantly high with mean concentrations of 3.9ppb and 2.0ppb in the sampled UCM and BCM respectively. The concentrations of all UCM samples and 10 sample of BCM were above the threshold of 0.5ppb accepted by the European union, the detection of high levels of AFm1 was above WHO permissible limits in most samples which calls for monitoring and control of AFM1. The high levels of AFM1 in the samples shows animal feeds being fed to cattle must have been polluted by Aflatoxin B1, the real precursor of AFM1.*

**Keywords:** *Aflatoxin M1, branded cow milk, unbranded cow milk.*

## 1. INTRODUCTION

Milk and its products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age people (Asadi *et al.*, 2012).Milk is a key contributor to improve nutrition and food security particularly in developing countries and play a significant role in reducing poverty and malnutrition (Kazemi *et al.*,2013).

It is a practice in Northern part of Nigeria and Some part of Africa that direct consumption of locallyprepared milk in villages is more frequent and acceptable as compared with consumption of pasteurized milk because it is a belief and cultural, that locally processed milk and its -products have more dietary benefits over the locally processed ones.(Younus *et al.*,2013).

Aflatoxins are mycotoxins which are group of naturally occurring toxins produced mainly by moulds such as *Aspergillusflavus and aspergillusparasiticus* and have adverse effects on humans, animals, and crops that result in illnesses and economic losses (Hussain and Anwar, 2008). These fungi contaminate wide range of agricultural products mainly cereal grains, during pre- and post-harvest stages (El Khoury*et al.*,2011). Factors like season, humidity, temperature and also drought in the farm and poor storage conditions have critical roles in production of Aflatoxins.$AFM_1$ is the hydroxylated metabolite of $AFB_1$and can be found in milk or milk products obtained from livestock that have ingested contaminated feeds (Arafa *et al.*, 2014).

## 2. MATERIAL AND METHODS

### 2.1. Sample Collection and Preparation

Cow milk from different markets (Railway market, Rido, Kasuwar Barchi, Zango Cattle Market, Kajuru, Panteka, Kasuwar Magani and Kaduna Central Market) was collected packed. Branded Cow Milk (BCM) were purchased across the counter at various supermarket in Kaduna metropolis. Each of the brand was purchased three times at different time interval and each with different batch number, (Farid 2010). The samples were collected in polyethylene bottles which were soaked in 20% $HNO_3$ for 24hours and rinsed with deionized water to avoid possible contamination. The name of each

Sample was replaced with an alphabet from A- Z for unbranded and A-O for branded. Names were followed by U meaning the product is Unbranded, or B meaning that the product is branded.

### 2.2. Sample Pretreatment

The samples were kept in a deep freezer ($-20^0$).Each label of the branded product was inspected to ensure proper marking and labeling information with emphasis on shelf life and batch number.

### 2.3. Extraction of Aflatoxin M1

A portion of 20ml of the sample was measured, the content was then transferred to as bottle with cover 25 ml of 70% Methanol was added followed by vigorous shaking at 250 rotations per minute (rpm) for 3 minutes in a horizontal shaker for the particulate matter to settle. 5 $cm^3$ of the extract underwent filtration using what man 1 filter paper and filtrate was collected for $AFM_1$analysis (Romer lab 2015)

### 2.4. Determination of AflatoxinM1

Two hundred (200 µl) of the conjugate solution was placed in each mixing well,100 µl sample extract was added to each dilution well containing the 200 µl of the conjugate solution, a dry multi channel pipette was used to thoroughly mixed the liquids in the wellone hundred (100 µl) of the solution was pipette and transferred to each corresponding antibody coated well and, the content of the mixing well was incubated at room temperature for fifteen (15) minutes, after incubation the content of the antibody wells were shaken out into a beaker and discarded, the antibody coated wells were then filled using distilled water and washed five times. the antibody coated well was taped to dry with adsorbent paper until the remaining water is removed.

One hundred (100 µl) of the substrate solution was then added into the antibody coated well and incubated at room temperature for 5 minutes, one hundred (100 µl) of stop solution was added into the antibody coated well, the change in color from blue to yellow indicate the presence of $AFM_1$.

The content was mixed by sliding back and forth on the flat surface; the bottom of the well was then wiped with a dry cloth. the absorbance was read within 20 minutes of the addition of the stop solution using Elisa at 450nM filter and 630nM differential filter, the result will be obtained and calculated in form of concentration using the ELISA stat fax micro well reader or equivalent (Romer labs 2015).

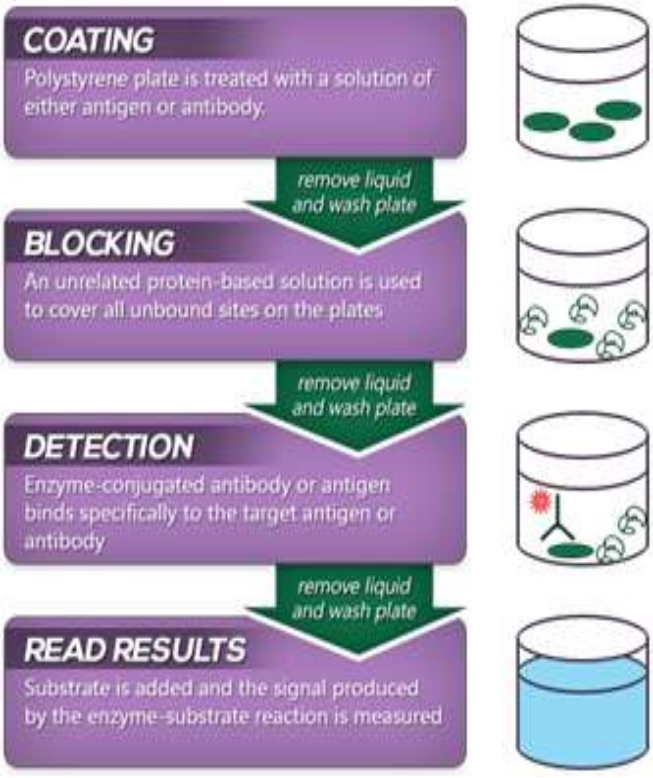


**Fig1.** *A summary of ELISA procedure*

## 3. RESULTS AND DISCUSSION

### 3.1. Concentration of Aflatoxin $M_1$ in Cow Milk Samples

Table 1.0 represent the mean concentrations of $AFM_1$from BCM, the mean concentrations of various branded product collected are within the range of 1.4-2.5 ppb with brand 4 having the highest contamination incidence and brand 1 having the lowest incidence of contamination, the values recorded on the mean concentrations were above the limit of 0.5 ppb as specified by European Union Standards. This may be attributed toingesting of crop residues contaminated by the fungi, their cows being hi- breed are mostly housed in a grazing space and mostly fed with processed and imported animal feeds which may likely be contaminated through poor storage conditions as well possibly on transit as some of the feeds were imported via sea port which make them vulnerable to moisture and water spillage exposure that facilitate the growth of the fungi.

Fig 2 shows the distribution of AFM in BCM, sample L-B of brand 4 has the highest value of 3.8 ppb while sample 3 of brand 1 and other 4 samples were within the limit of acceptable value of 0.5 ppb this may be attributed to the cattle being fed with feed that is not contaminated with AFB.

Fig 4 shows comparison of the result obtain with EU guidelines, it indicates75% contamination incidence, 10 of the samples were at unsafe levels while the other five at safe level. Similar report was shown by Makun *et al.,* (2016) in the determination of $AFM_1$ in breast Milk, Cow Milk and Milk products in Minna, it is also similar with report of Asia *et al.* (2011) and Bilandžić *et al.*(2014) in Croatia.

**Table1.** *Concentrations (ppm) of $AFM_1$ in BCM*

| Brand | Sample label | Concentrations (ppb) | Mean concentrations(ppb) ± SD |
|---|---|---|---|
| 1 | A-B<br>B-B<br>C-B | 1.0<br>1.0<br>2.1 | 1.8 ± 0.8 |
| 2 | D-B<br>E-B<br>F-B | 2.0<br>3.0<br>0.3 | 1.8± 3.7 |
| 3 | G-B<br>H-B<br>I-B | 3.2<br>2.9<br>0.4 | 2.2± 4.7 |
| 4 | J-B<br>K-B<br>L-B | 0.5<br>3.2<br>3.8 | 2.5± 2.5 |
| 5 | M-B<br>N-B<br>O-B | 3.5<br>0.5<br>2.2 | 2.1± 2.1 |

From table 1 below Samples from Railway market has the highest contamination incidence of $AFM_1$ with mean concentration of 5.6 ppb and the lowest contamination incidence was with mean concentration of 2.2 ppb recorded from samples collected from Panteka, Fig 3 shows the mean concentrations of $AFM_1$in UCM with samples from Railway market recording highest incidence and sample from Panteka recording lowest incidence. However all the values recorded on the concentrations were above the limit of 0.5 ppb as specified by European Union Standard. This may be attributed to ingesting of crop residues contaminated by the fungi and left over after harvesting in the farms where animals graze freely uncontrollable and unrestricted (Makun *et al*., 2016). Another source of contamination of the feed may be poor storage condition of the feed which expose it to high humidity, drought and poor storage temperature which exposes the feed to attack by molds/ fungi that produces AFB which get converted to $AFM_1$ through hydroxylation in the liver(Melkamu and Birham, 2013). From the result of this investigation $AFM_1$ in UCM in Kaduna is similar to report of Makun *et al.*,(2016) in the determination of $AFM_1$ in breast Milk, Cow Milk and Milk products in Minna, it is also similar to the report of Okeke *et al.*,(2012)in the preliminary survey of $AFM_1$in dairy Cattle products in Bida Niger State Nigeria, it is also similar to the report of Hussaini and Anwar,*(*2008) in study on contamination of in raw milk in the Punjab province of Pakistan. it is equally similar Younus *et al.*,(2013) in Jhang city of Pakistan. Higher concentrations were however reported by Oluwafemi *et al.,* (2014) in the Survey of $AFM_1$ in cows 'milk from free grazing cows in Abeokuta Nigeria.

**Table2.** *Concentrations (ppm) of $AFM_1$ in UCM*

| Sample location | Sample label | Concentrations (ppb) | Mean concentrations(ppb)± SD |
|---|---|---|---|
| Panteka | A-U<br>B-U<br>C-U | 2.5<br>2.2<br>1.8 | 2.2± 0.5 |
| Railway market | D-U<br>E-U<br>F-U | 1.0<br>7.8<br>8.1 | 5.6± 3.5 |
| Rido Ranch | G-U<br>H-U<br>I-U | 1.6<br>1.9<br>2.3 | 2.5± 1.1 |
| KasuwarBarchi | J-U<br>K-U<br>L-U | 1.9<br>1.2<br>8.1 | 3.7± 0.7 |
| Zango Cattle Market | M-U<br>N-U<br>O-U | 8.2<br>1.6<br>1.9 | 3.9± 4.9 |
| Kajuru Ranch | P-U<br>Q-U<br>R-U | 2.3<br>3.8<br>4.5 | 3.5± 1.6 |
| Kasuwar Magani Ranch | S-U<br>T-U<br>W-U | 3.6<br>3.5<br>3.7 | 3.6± 1.6 |
| Abubakar Gummi Market | X-U<br>Y-U<br>Z-U | 1.9<br>1.2<br>8.1 | 3.7± 0.7 |

### 3.2. Comparison of Levels of Aflatoxin $M_1$ in UCM & BCM

This investigation indicates that the unbranded milk have higher incidence of contamination of $AFM_1$, this may be attributed to freely and unrestricted grazing of cattle which made them vulnerable to ingesting feed that is contaminated with $AFB_1$.Which is due to poorpost-harvest practice that expose the crops to mould and fungi and subsequent production of Aflatoxins $B_1$. (Makun *et al.*, 2016).Milk from cows that freely graze without proper monitoring and control had the highest concentration of $AFM_1$ (100%). In Nigeria, the sources of Aflatoxin contamination are well-defined and discussed (Makun*et al.*, 2016). A major factor is lack of good warehousing practice of the harvested crops and poor post harvest practice that expose the crops to mould and fungi and subsequent growth of Aflatoxins. Groundnut and corns are found to be common source of Aflatoxin $B_1$ contamination (Makun *et al.*, 2016).

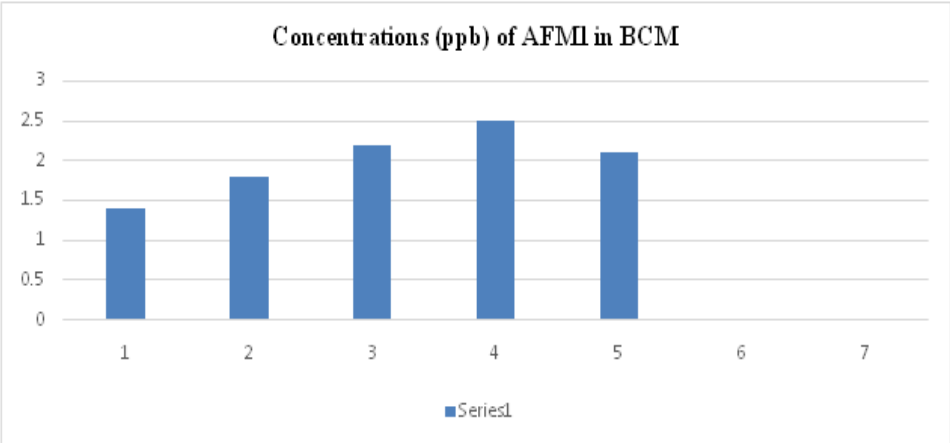


**Fig2.** *Concentrations (ppb) of AFM1 in BCM*

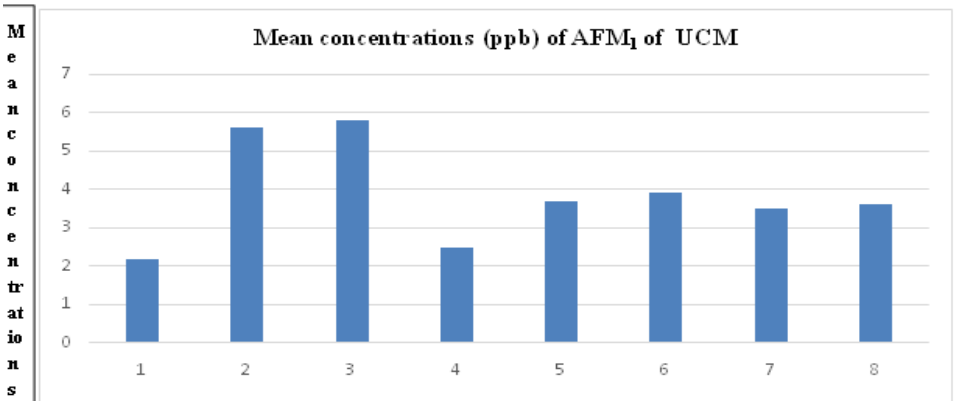


**Fig3.** *Mean concentrations (ppb)of $AFM_1$ of UCM*

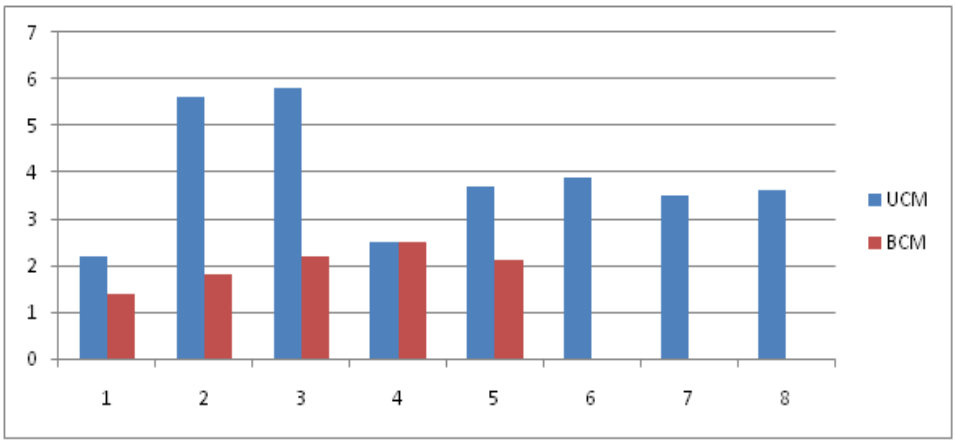


**Fig4.** *Comparison of mean concentrations (ppb) of BCM and UCM*

## 4. Conclusion

Finding reported in this study indicates contamination of the various brands of BCM samples with 75% contamination incidence recorded. (10 of 15 brands contains $AFM_1$) the values were above the limit specified by EU/FAO. In UCM, all samples collected within Kaduna metropolis were found to be above specifications of EU/FAO therefore highly unsafe for consumption. Consumption of feed infected by Aflatoxin leads to different problems in reproductive, digestive and respiratory tracts of livestock causing infected milk production. Consumption of infected milk by human incurs major hygienic and pharmaceutical costs to society. Therefore, in order to prevent from introduction of Aflatoxin M1 into food industry cycle, its precursor namely Aflatoxin B1 should be controlled. To obtain this, meeting hygienic conditions, appropriate storage and control of livestock feed at all stages of planting, growing, harvesting, producing and storing are necessary. For this reason, milk and milk products have to be controlled continuously by accurate and reliable analytical techniques for presence of AFM1 contamination. It is also extremely important to maintain low levels of AFM1 in the feeds of dairy animals. In order to achieve this, dairy cow feds should be kept away from contamination as much as possible. Therefore, animal feeds should be checked regularly for Aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled. The regulatory limits are widely variable and there has been little scientific basis in their setting. Efforts should be made in attempting to provide further and extensive scientific information on human health hazards related to low-level long term Aflatoxin exposure and to standardize the already existing regulatory limits for Aflatoxin. .

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