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# Evaluation of Aflatoxin $M_1$ Residues in Cow's Milk Sold in the Communes of Ouagadougou and Dedougou (Burkina Faso)

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**Abstract:** Aflatoxins $B_1$ mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Indeed, aflatoxins $B_1$ ($AFB_1$) are mycotoxins that can contaminate a wide variety of foods. The ingestion of food contaminated with farm animals may result in the alteration of their health and zootechnical performance as well as a food safety problem related to the presence of aflatoxin $M_1$ residues ($AFM_1$) in the products animals, especially milk. In humans, aflatoxins, especially $AFM_1$, found mostly in milk, have a hepatotoxic, carcinogenic, immunotoxic effect and also impair the functioning of reproductive organs. It is for this reason that the present study was initiated to evaluate the residues of aflatoxin $M_1$ in the cow's milk sold in the communes of Ouagadougou and Dedougou. A collection of 16 and 20 samples was carried out respectively in the cities of Dedougou and Ouagadougou. Chromatographic analysis by HPLC of our samples showed an absence of aflatoxin $M_1$ in both localities. A comparison of our results with the standard set by the European Commission (EC) shows that our samples have good quality. These results could be justified by the good quality of cow's food. In view of these results, farmers should be encouraged to adopt and continue a healthy diet of draft cows for a better valorization of local milk.

**Keywords:** Cow's Milk, Aflatoxin $B_1$, Aflatoxin $M_1$ Residues

## 1. Introduction

In a global market context, food security is receiving increased attention from consumers, governments and producers due to awareness of human and animal health issues. In general, incidents related to food chain contamination provoke strong media coverage that reinforces concerns [1]. In particular, the presence of mycotoxins in foods of plant or animal origin such as aflatoxins in dairy products, due to their widespread consumption poses a serious public health problem [2]. In humans, aflatoxins, especially $AFM_1$, found mostly in milk, have a hepatotoxic, carcinogenic, immunotoxic effect and also impair the functioning of reproductive organs [3].

Indeed, these contaminants ($AFM_1$) are found indirectly in milk through animals. Also, some studies show that the diet (cakes) of ruminants is a source of aflatoxin [4]. These ruminants can metabolize aflatoxins ($AFB_1$) contaminating their food with $AFM_1$ and thus release it into milk. Aflatoxin $M_1$ ($AFM_1$) is thus a major metabolite of aflatoxin $B_1$ ($AFB_1$), which is formed when animals ingest food contaminated with aflatoxin $B_1$.

In addition, Burkina Faso has a large digital potential for cow's milk, with total production estimated at 2, 691,092 liters in 2009 and 250 million liters in 2018 [5]. This covetousness of milk by the population is related to its nutritional intake of primary metabolites (carbohydrates, proteins, lipids, minerals and vitamins)

hence its full food name. Beyond these nutritional properties, toxicological evaluation is an important indicator of the quality of local milk for its recovery. However, very little data exist on the toxicological contamination of milk including $M_1$ aflatoxins.

It is in this context that our study aims to evaluate the residues of aflatoxin $M_1$ in fresh cow's milk sold in the communes of Dedougou and Ouagadougou. Specifically, it was for us to detect aflatoxin $M_1$ residues in the collected samples, quantify aflatoxin $M_1$ residues in the collected samples and to assess the risk of aflatoxin $M_1$ contamination based on the study area.

## 2. Materials and Methods

### *2.1. Biological Material*

The biological material was essentially cow's milk collected in the communes of Ouagadougou and Dedougou precisely in the cities of Ouagadougou and Dedougou.

Study area

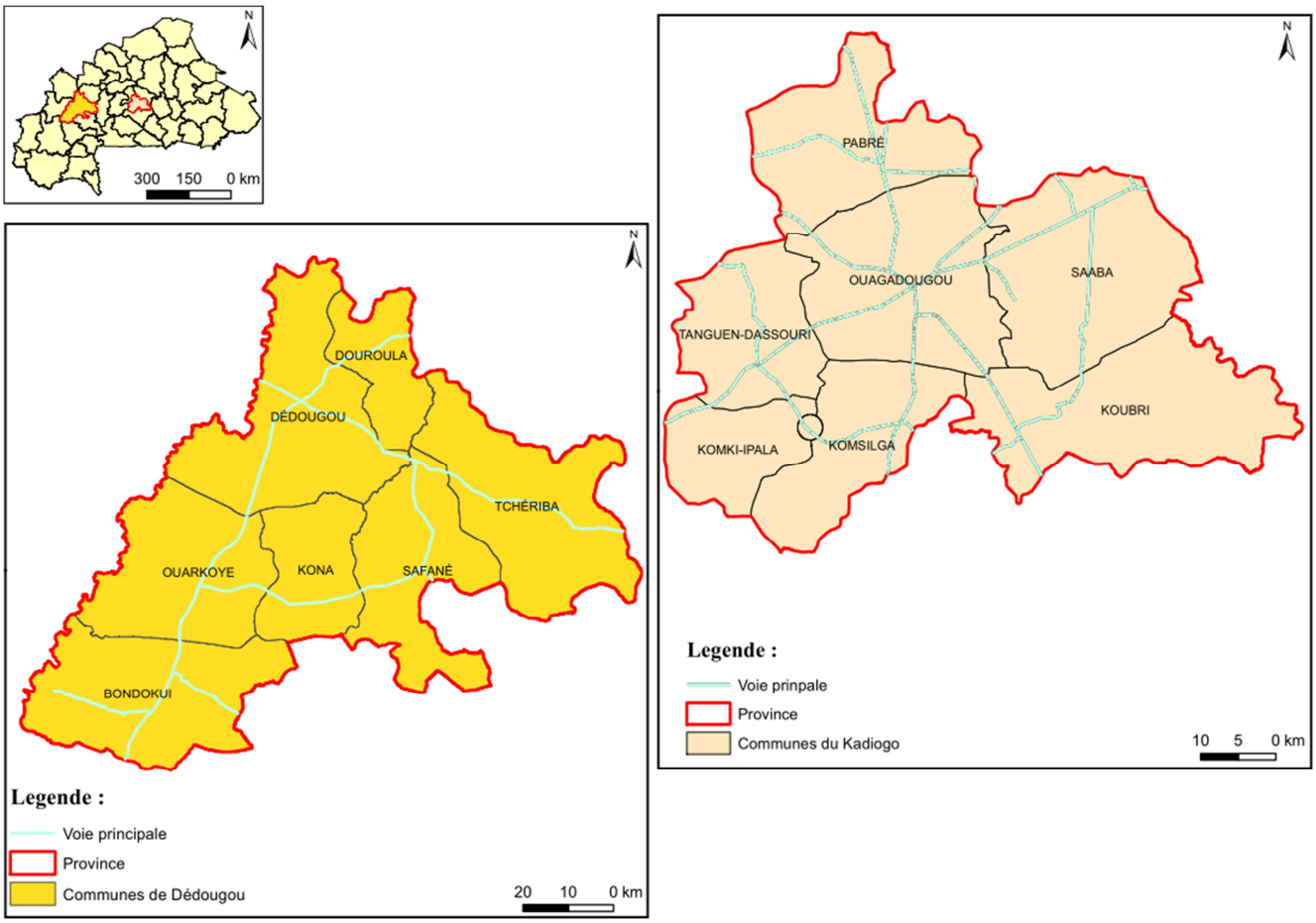


***Figure 1.*** *Map of the study area [6].*

### *2.2. Sampling*

The collection of samples took place from August 27 to September 28, 2019. We collected a total of 36 samples including 16 samples in the commune of Dedougou and 20 samples in Ouagadougou. For the commune of Dedougou, the collection was made from farmers and milk sellers while for that of Ouagadougou, it was carried out in the markets of Hamdallaye, Zogona, Katr-yaar and zone one and in a supermarket. The samples were placed in sterile vials and packaged in a thermostable cooler at -4°C and then transported directly to the Laboratory on the same day.

***Figure 2.*** *Milk samples contained in the vials [7].*

### *2.3. Aflatoxin $M_1$ Assay Methods*

Aflatoxin $M_1$ was assayed by Thermo Ultimate 3000 High Performance Liquid Chromatography (HPLC) with a

pressure of up to 1000 bar [8].

Prince: Extraction of aflatoxin $M_1$ from a milk sample is performed by passing the milk sample through an immuno-affinity column. The column contains specific antibodies attached to a solid support. As the sample passes through the column, the antibodies selectively bind to the $M_1$ aflatoxin (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are removed from the column by distilled water. Then aflatoxin $M_1$ is eluted from the column and the eluate is collected. The amount of aflatoxin $M_1$ present in this eluate is determined by Higth Performance Liquid Chromatography (HPLC), followed by fluorimetric detection.

Extraction: Using 02 Falcones tubes with a capacity of 50 ml each, take 100 ml of milk and centrifuge at a radial acceleration of 4000 rpm for 15 minutes and then filter the milk through the Whattman filter paper so that the fat can be removed. Let's collect 50 ml of the filtered milk and then vortex the filtrate obtained.

Purification: Purification of $AFM_1$ is performed by passing 50 ml of the filtrate through an immuno-affinity column. The column contains specific antibodies attached to a solid support. Afterwards, all other components of the sample matrix are removed from the column with distilled water (2 × 10ml). Then we change the exhaust pipe. Then, the desired aflatoxin is eluted from the column with 1500 µl of acetonitrile and 500 µl of distilled water. Finally, the eluent is transferred to vials that will be placed on the injection portion of the HPLC with fluorescence detector.

Chromatographic conditions: The High Performance Liquid Chromatographic (HPLC) chain used is branded Agilent Technology 1100 series consisting of the following modules such as isocratic pump with regular flow, injection system with a fixed volume, analytical column for reverse phase polarity chromatography lined with 3 µm or 5 µm octadecycle silica gel and with a guard column filled with reverse phase polarity materials (C18) and analysis was carried out during 8 min.

Mobile phase: Solvent for mobile phase of HPLC ($H_2O$: 75V; ACN: 25 V → 1000 ml. 750 ml of distilled water is taken, 250 mL of acetonitrile is added.)

Expression of results: The $AFM_1$ content of the sample in µg/l of the sample can be calculated using the following formula:

$$C = m_A \times \frac{v_f}{v_i x v}$$

Where:

C is the concentration of aflatoxin M1 in µg/Kg;

$m_A$ is the numerical value of the mass of aflatoxin M1, expressed in ng;

$v_i$ is the volume of sample extract injected in µl;

$v_f$ is the final volume of the sample extract eluted in µl;

V is the volume of the sample for the test sample in ml.

# 3. Results and Discussions

### *3.1. Results*

Figure 3 gives the curve of white. This curve shows that the solution used to assay our samples is not contaminated with $AFM_1$. She serves as a witness. Figure 4 is a curve which shows the peak of $AFM_1$ which comes out at 8 minutes. Figure 5 is a standard calibration curve. After analysis, the curve of each sample is compared to the curve of the standard. None of the samples can be seen to peak at 8 minutes. This would mean that the $AFM_1$ content in our samples is below the optimal detection limit of 0.008ppb. These results show that these products are safe for human consumption.

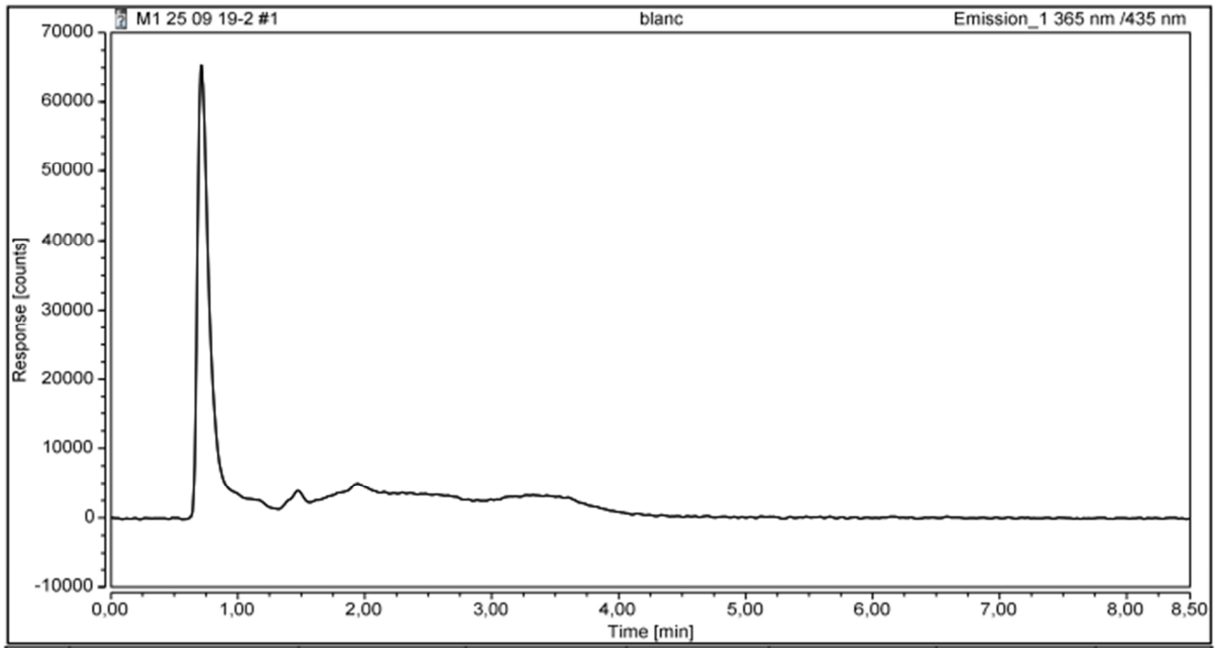


***Figure 3.*** *Chromatogram of blank.*

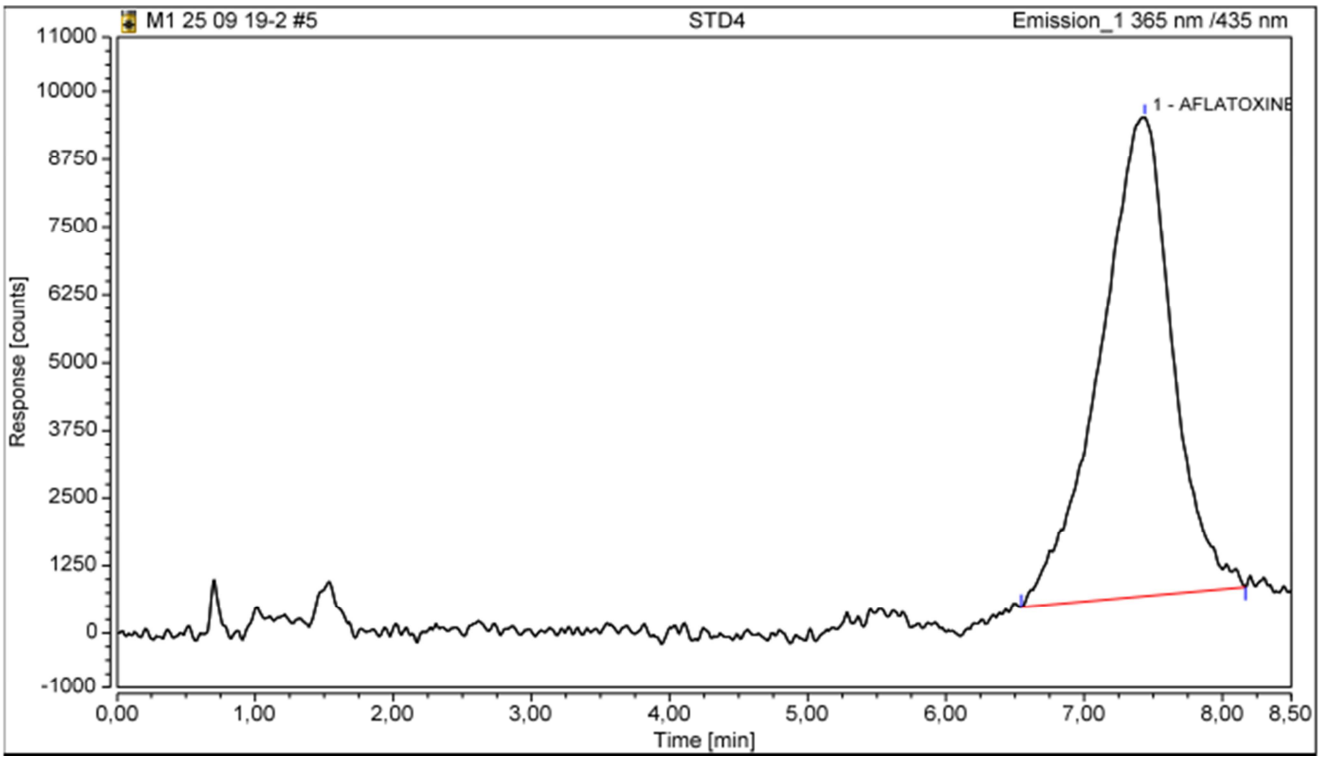


***Figure 4.*** *Chromatogram of Standard 4.*

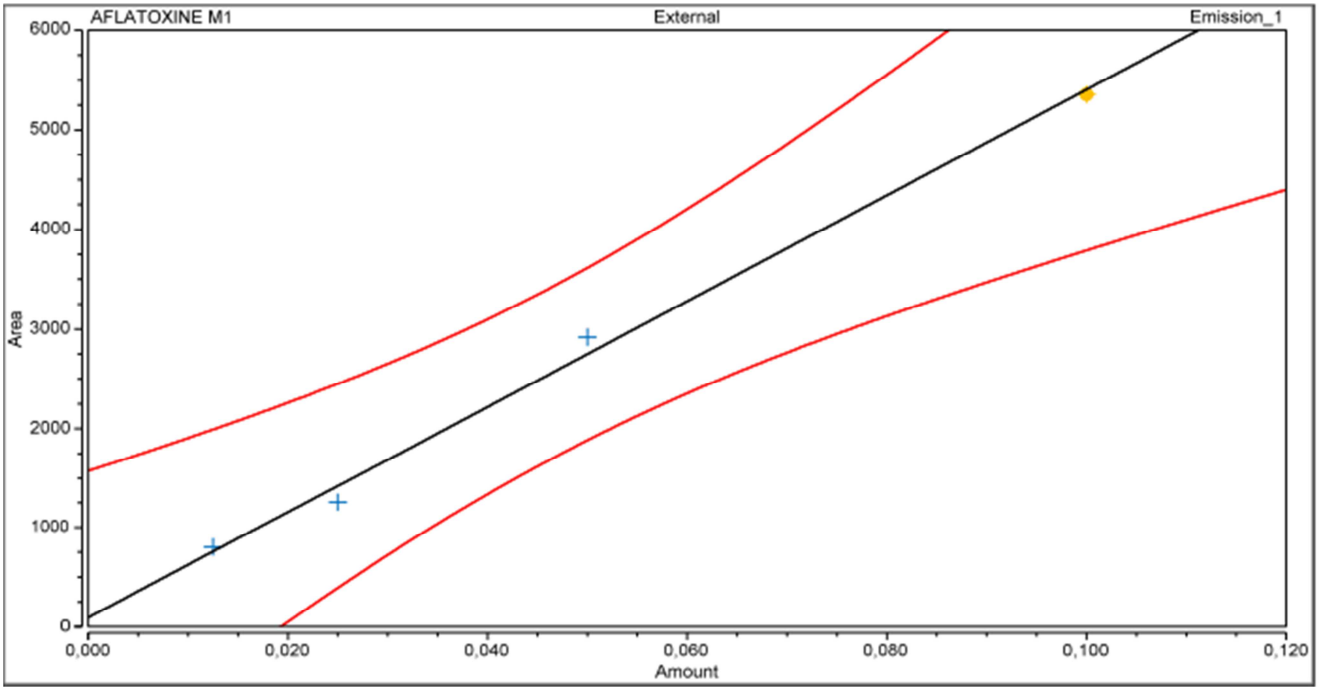


***Figure 5.*** *The standards calibration curve.*

Curve as described by the following regression equation: $Y = 29.77x - 3.75e^{-1}$. 1, where y=area and x=amount of $AFM_1$. The results showed the linearity of the standard curve over the range studied. The coefficient of determination ($R^2$) was 0.9953.

### *3.2. Discussions*

Analysis of our results showed that none of our samples were contaminated with $AFM_1$. Our results are in agreement with those of [9, 10] who analyzed samples of raw cow's milk. However, none of these samples exceeded the maximum limit authorized by the European Commission which is 0.025 µg/kg for infants and 0.05 µg/kg for adults. In addition, our results corroborate surveys carried out in Europe whose results revealed that the incidence of $AFM_1$ contamination is much lower (~ 1%) [11, 12].

Unlike our results, [13] in Kuwait, [14] in Iran, [15] in Lebanon found 36%, 59% and 60.7%, respectively, of samples contaminated with $AFM_1$, exceeding the European standard (50 ng/l). In these studies, the diet of dairy cows consisted mainly of fodder, cake, corn silage, etc. These results would be justified by the physico-chemical conditions (water activity, substrate, pH, etc.) of the medium. Indeed, our samples were all taken after the winter period (August for the samples from Dedougou and September for those from Ouagadougou), when the ration of the dairy cows consisted mainly of pasture which is generally of good quality because the Rainwater leaches the pasture and carries away certain microorganisms. This period also does not favor the development of certain molds such as *Aspergillus flavus* and *Aspergillus parasiticus*, which are unable to secrete toxins on grasses and grazing plants. In fact, the quantity of $AFM_1$ found in milk is correlated with the level of contamination of food consumed by dairy animals. Aflatoxin $M_1$ levels correspond approximately to 1.7% of the $AFB_1$ concentration present in the food ingested and depend on the physiological and metabolic state of the animal [16]. In view of the above, we can say on the one hand that our dairy cows have ingested a low content of $AFB_1$ such that their metabolite ($AFM_1$) cannot be found in the milk. On the other hand, the level of $AFM_1$ present in our samples is below the optimal limit of quantification. The optimum limit of quantification is 0.008 ppb.

Analysis of our results also reveals that no significant difference exists between our samples depending on the sampling area. This analysis could be justified by the similarity of breeding techniques. Indeed, a survey conducted with the "SOUDOU-KOSSAM" dairy reveals that the breeders of Dedougou in the Boucle du Mouhoun apply good breeding practices because they are constantly monitored and controlled by the livestock management of the said locality. In addition, these good breeding practices could be one of the reasons for the non-contamination of our samples. To this can be added our experimental conditions and the number of our samples which is not sufficiently representative compared to the number of dairy cows that exist in Burkina Faso.

# 4. Conclusion

The objective of this thesis was to assess the level of $AFM_1$ aflatoxin residues in fresh milk collected in Dedougou and Ouagadougou. At the end of our work, it appears that our samples do not contain quantifiable aflatoxin residues. Then we can also note that the collection area did not have a negative impact on the contamination of our samples with aflatoxin. These results could be justified by the low content or absence of aflatoxin in the diet of our cows. In view of these results, consumption of these cow's milk presents a low risk of contamination for humans. However, it should be noted that this study is only a contribution to the evaluation of $AFM_1$ in cow's milk and cannot reflect the real situation across Burkina Faso.

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