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# The presence of aflatoxin M<sub>1</sub> on processed milk in the Republic of Kosovo

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**Abstract** Aflatoxins are mycotoxins of major concern to the dairy industry. Given the fact that aflatoxins M<sub>1</sub> mycotoxins can cause toxic effects called mycotoxicosis. It has been reported that mycotoxins are carcinogenic, tumorigenic, and dermatological in a large number of organisms and cause hepatic carcinoma in humans. This study aims to identify the quantity of mycotoxins M<sub>1</sub> in dairy products used by consumers in the Republic of Kosovo. According to the results obtained from the analyzes of processed milk samples we have come to the conclusion that the content of aflatoxin M<sub>1</sub> in all types of dairy products is not allowed limits according to the regulation in force for the safety of food products.

**Keywords:** Mycotoxins, Aflatoxins M<sub>1</sub>, Carcinogenic, Tumorigenic

## INTRODUCTION

A very large number of molds produce toxic substances designated mycotoxins. Some are mutagenic and carcinogenic, some display specific organ toxicity, and some are toxic by other mechanisms. Mycotoxins are produced as secondary metabolites. The primary metabolites of fungi as well as for other organisms are those compounds that are essential for growth. Secondary metabolites are formed during the end of the exponential growth phase and have no apparent significance to the producing organism relative to growth or metabolism. In general, it appears that they are formed when large pools of primary metabolic precursors such as amino acids, acetate, pyruvate, and so on, accumulate. The synthesis of mycotoxins represents one way the fungus has of reducing the pool of metabolic precursors that it no longer requires for metabolism.<sup>1</sup>

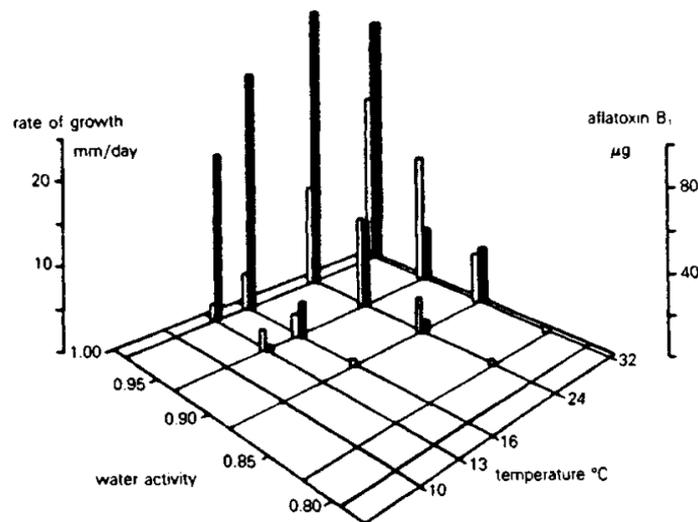
Aflatoxins are clearly the most widely studied of all mycotoxins. Knowledge of their existence dates from 1960, when more than 100,000 turkey poults died in England after eating peanut meal imported from Africa and South America. From the poisonous feed were isolated *Aspergillus flavus*, and a toxin produced by this organism that was designated aflatoxin (*Aspergillus flavus* toxin-A-fla-toxin). Studies on the nature of the toxic substances revealed the following four components:

It was later determined that *A parasiticus* produces aflatoxins. Another *Aspergillus* species, *A. nominus*, also produces aflatoxins<sup>2</sup> these compounds are highly substituted coumarins, and at least 18 closely related toxins are known. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is produced by all aflatoxinpositive strains, and it is the most potent of all. AFM<sub>1</sub> is a hydroxylated product of AFB<sub>1</sub> and appears in milk, urine, and feces as a metabolic product.<sup>3</sup> AFL, AFLH<sub>1</sub>, AFQ<sub>1</sub>, and AFP<sub>1</sub> are all derived from AFB<sub>1</sub>. AFB<sub>1</sub> is the 2, 3-dehydro form Of AFB<sub>1</sub>, and AFG<sub>1</sub> is the 2, 3-dihydro form of AFB<sub>1</sub>. The toxicity of the six most potent aflatoxins decreases in the following order: B<sub>1</sub> >

M1 > G1 > B2 > M2 ≠ G2.<sup>4</sup> When viewed under ultraviolet (UV) light, six of the toxins fluoresce as noted:

B<sub>1</sub> and B<sub>2</sub>—blue; G<sub>1</sub>—green; G<sub>2</sub>—green-blue; M<sub>1</sub>—blue-violet; M<sub>2</sub>—violet

No aflatoxins were produced by 25 isolates of *A. flavus/parasiticus* on wort agar at 2°, 7°, 41°, or 46°C within 8 days, and none was produced under 7.5° or over 40°C even under otherwise favorable conditions.<sup>5</sup> In another study employing Sabouraud's agar, maximal growth of *A. flavus* and *A. parasiticus* occurred at 33°C when pH was 5.0 and water activity (a<sub>w</sub>) was 0.99.<sup>6</sup> At 15°C, growth occurred at a<sub>w</sub> 0.95 but not at 0.90, while at 27° and 33°C, slight growth was observed at an a<sub>w</sub> of 0.85. The optimum temperature for toxin production has been found by many to be between 24° and 28°C. In one study, maximal growth of *A. parasiticus* was 35°C, but the highest level of toxin was produced at 25°C.<sup>7</sup> The limiting moisture content for AFB<sub>1</sub> and AFB<sub>2</sub> on corn was 17.5% at a temperature of 24°C or higher, with up to 50 ng/g being produced.<sup>8</sup> No toxin was produced at 13°C. Overall, toxin production has been observed over the a<sub>w</sub> range of 0.93 to 0.98, with limiting values variously reported as being 0.71 to 0.94.<sup>9</sup> In another study, no detectable quantities of AFB<sub>1</sub> were formed by *A. parasiticus* at a<sub>w</sub> values of 0.83 and 10 °C.<sup>10</sup> The optimum temperature at a<sub>w</sub> 0.94 was 24°C (Figure 1). Growth without demonstrable toxin appeared possible at a<sub>w</sub> 0.83 on malt agar-containing sucrose. It has been observed by several investigators that rice supports the production of high levels of aflatoxins at favorable temperatures but none is produced at 5 °C on either rice or cheddar cheese.<sup>11</sup>



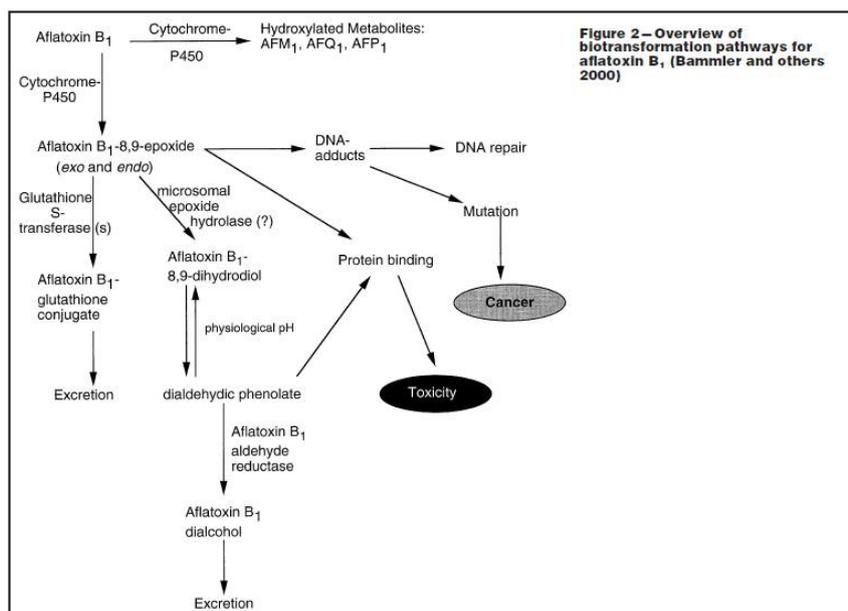
**Figure 1** Growth and aflatoxin B<sub>1</sub> production on malt extract-glycerine agar at various water activity values and temperatures. White columns: rate of growth; black columns: average AFB<sub>1</sub> production. Source: From Northolt et al.,<sup>10</sup> copyright © 1976 by International Association of Milk, Food and Environmental Sanitarians.

Overall, the minimal and maximal parameters that control growth and toxin production by these eukaryotic organisms are not easy to define, in part because of their diverse habitats in nature and in part because of their eukaryotic status. It seems clear that growth can occur without toxin production.

AFG<sub>1</sub> is produced at lower growth temperatures than AFB<sub>1</sub>, and while some investigators have found more AFB<sub>1</sub> than AFG<sub>1</sub> at around 30 °C, others have found equal production. With regard to *A. flavus* and *A. parasiticus*, the former generally produces only AFB and AFG.<sup>12</sup> Aeration

favors aflatoxin production, and amounts of 2 mg/g can be produced on natural substrates such as rice, corn, soybeans, and the like.<sup>11</sup> Up to 200 to 300 mg/L can be produced in broth containing appropriate levels of Zn<sup>2+</sup>. The release of AFB<sub>1</sub> by *A. flavus* appears to involve an energy - dependent transport system.

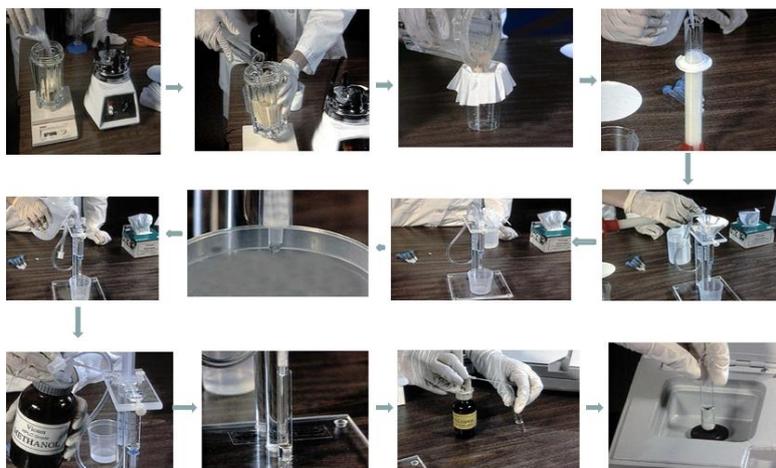
Aflatoxins are a group of structurally related mycotoxin which consist of four naturally occurring compounds including aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> which are mainly produced by three species of moulds namely *Aspergillus flavus*, *A. parasiticus* and rarely *A. nomius*.<sup>13</sup> Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) has strong teratogenic, mutagenic, and carcinogenic effects.<sup>14</sup> Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a monohydroxylated derivate of AFB<sub>1</sub>, which in liver is metabolized by cytochrome *P450* Figure 2. It is excreted into the milk of both human and lactating animals.<sup>15</sup> A direct relationship has been shown between the amount of AFB<sub>1</sub> consumption and the secreted AFM<sub>1</sub> in milk. It has been shown that in 0.3-6.2% consumed AFB<sub>1</sub> by animals is secreted into milk.<sup>16</sup> The content of AFM<sub>1</sub> in milk is not significantly affected by thermal process, pasteurization and ultra-high-temperature (UHT) treatment, or storage of dairy products.<sup>17</sup> Although AFM<sub>1</sub> is less carcinogenic and mutagenic than AFB<sub>1</sub> as its parental compound,<sup>18</sup> but the international agency for research on cancer (IARC, 2002)<sup>19</sup> has re-categorized it from group 2 to group 1 according to recent investigations on its carcinogenicity.



Milk and dairy products constitute an important part of people's diet providing as a good source of calcium and proteins. Therefore, presence of aflatoxins in these products poses an important hygienic risk in human health. Considering its public health significance, many countries have set maximum limits for aflatoxins, which vary among countries. European Community (EC) has prescribed the maximum acceptable level of AFM<sub>1</sub> in liquid milk as 50 ng/l (EC, 2001). Both, US food and drug administration (FDA, 1996)<sup>20</sup> and institute of standards and industrial research of Iran (ISIRI, 2005)<sup>21</sup> have set the maximum level of 500 ng/l of AFM<sub>1</sub> in liquid milk. This study was aimed to determine the levels of AFM<sub>1</sub> in pasteurized and UHT milk marketed in West-Azerbaijan province as a main dairy producing region in Iran.

## MATERIALS AND METHODS

For the identification of mycotoxins  $M_1$  in UHT milk, the fluorometric method with automated VICAM Series 4 Fluorimeter system was used. A total of 30 samples were analyzed for each different milk percentage (3.2%, 3.5%, 3.8%) analyzed by 10 samples. The analyzes were carried out in the accredited Laboratory of the Food and Veterinary Agency, Pristina. Figures 3 and 4 show schematic sampling procedures and the automatic setup for identifying  $M_1$  mycotoxins.



**Figure 3.** Procedures to be followed for sample preparation for the VICAM automatic system

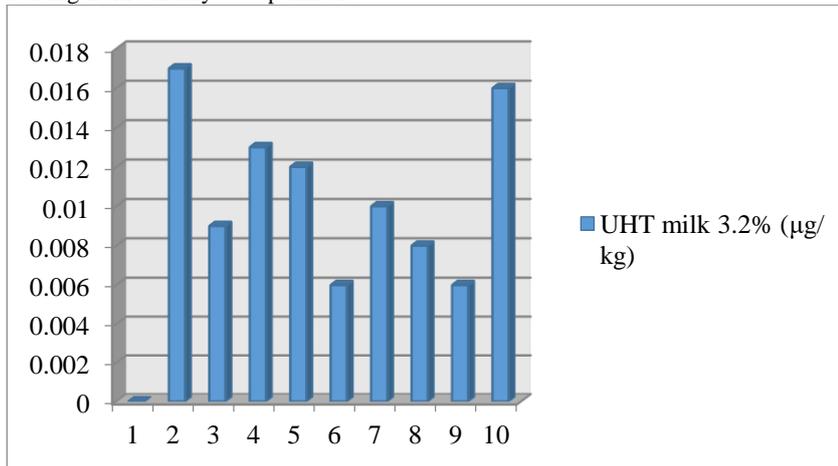


Series 4 Fluorimeter  
Field Service Manual

**Figure 4.** Automatic system VICAM

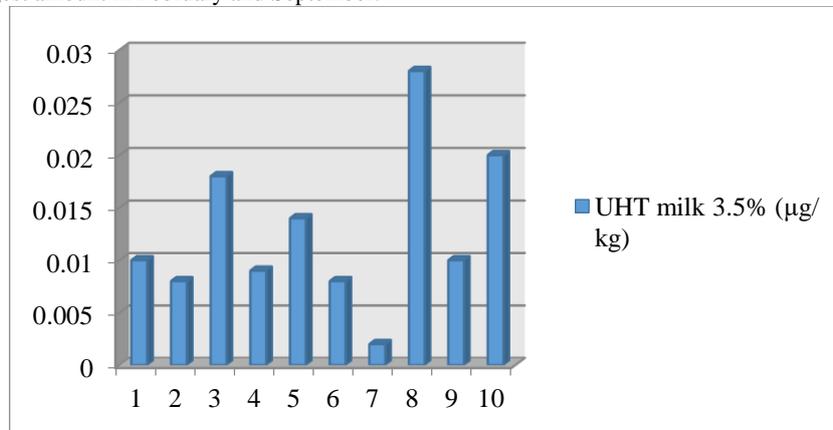
## RESULTS E DISCUSSION

The results obtained in this study are shown in graphs 5, 6 and 7 where they are analyzed by 10 samples for each milk with different fat percentages. The analyzes are carried out every month starting from January to September.



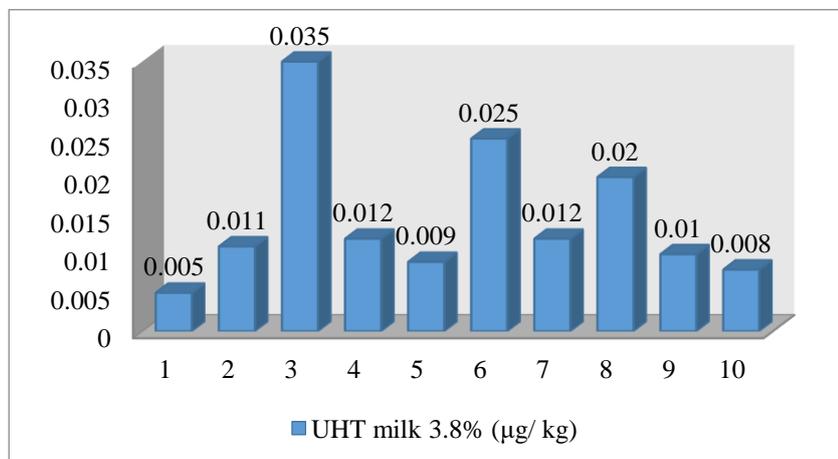
Graph 5.

The results reflected in graph 5 show a smaller amount of mycotoxin M<sub>1</sub> in January, while the largest amount in February and September.



Graph 6.

In the graph no. 6 is the lowest amount of mycotoxin M<sub>1</sub> in July, and the highest amount in August.



**Graph 7**

In the graph no. 7 where the results of mycotoxin M<sub>1</sub> are reported is the lowest amount of mycotoxin M<sub>1</sub> in January, while the highest amount in March.

The average values of mycotoxins M<sub>1</sub> for milk with different percentages are these:

1. Milk with 3.2% fat 0,010 µg / kg,
2. Milk with 3.5% fat 0.012 µg / kg and
3. Milk with 3.8% fat 0.009 µg / kg.

## CONCLUSION

Based on the results obtained during this study we can conclude:

1. Milk taken in the study for the period of time meets the safety standards since all the samples taken in the study are below the critical limit set in the regulation 0,05 µg/ kg, There is an increase in the amount of mycotoxins M<sub>1</sub> after the month of February, Control of animal foods to prevent the consumption of aflatoxin B<sub>1</sub> and the prevention of mycotoxins M<sub>1</sub> in milk.  
Regular control of fresh milk for the presence of aflatoxins M<sub>1</sub>.

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