**OCCURRENCE OF AFLATOXIN B1 IN FEED MATERIAL AND AFLATOXIN M1 IN GOAT MILK IN THE AREA OF NORTH-WEST SERBIA**

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**ABSTRACT**


AFB1 is one of the most toxic known mycotoxin. Its metabolite AFM1 is made from AFB1 in liver by the CYP 450 and excreted in milk, feces and urine. Some papers give estimation that AFM1 is 10% carcinogenic and toxicogenic from its precursor AFB1, it is classified as a possible carcinogenic (Class 2B) by the International Agency for Research on Cancer (1993). The European regulatory limit for AFM1 from the 1999, in milk is 0,05μg/l. This regulatory limit is necessary for protection of consumers, especially younger population, who is the most often consumers of milk and milk products.

From January 2007 to May 2007, 18 samples of feed and raw goat milk, from different individual farms in Serbia are analyzed on occurrence of aflatoxin B1 in feed and aflatoxin M1 in milk. In 8 of the 18 samples of feed (44.44%) the presence of AFB1 was above EU regulatory limit for aflatoxin B1 in feed (5 μg/kg). The incidence rates of AFM1 with concentration from 0,0-0,125 µg/l was 33,3% (6 samples), with concentration 0,0125-0,05 µg/l was 27,8% (5 samples), and number of samples with exited level from the European limit regulation was 38,9% (7 samples).

**Key words**: aflatoxin B1, aflatoxin M1, goat milk

**Introduction**

The accumulation of mycotoxins in foods and feeds represents a major threat to human as they are responsible for many different chronic health risks, including the induction of cancer, and digestive, blood and nerve defects. Mycotoxins negatively impact agriculture and associated industries, in different ways, in all parts of the globe. The economic consequences of mycotoxin contamination are profound, and often crops with large amounts of mycotoxins have to be destroyed.

Aflatoxins are large group of mycotoxins, one of members of this group aflatoxin B1 is the most toxic and also most often investigated, it is classified as a potent carcinogen (class A) (IARC, 2002). It is first discovered in 1960s when caused lot damage on one farm in England (Blount, 1961). Aflatoxins are mycotoxins produced by molds from the genus Aspergillus, most often by *A. flavus* and *A. para-sicus*, but can be produced by another species from the genus Aspergillus, like *A. tamarii* or *A. nomius* (Goto et al. 1996, Henry et al., 2004). Aflatoxins are produced pre and post harvest period under certain condition of temperature moisture, and availability of nutrient in feed material originated from subtropics and tropic region of word. The main source of aflatoxin B1 in feed are cotton seed, maize, peanut meal. Aflatoxin B1 in feed material can be fined occasionally in some part of Europe (some report of high contaminated maize in north Italy (EFSA, 2003, RASFF, November 2003).

Aflatoxin M1 is metabolite of aflatoxin B1, and it is excreted in milk, feces and urine of animals which consumed feed with aflatoxin B1, it is classified as a possible carcinogen (Class 2B) (IARC, 1993). Carry over from feed to milk vary greatly, depending from condition, health status of animals and period of lactation. The greatest carry over was found in milk of cows with high productions of milk.
from 2.6 to 6.2 % (Veldman et al., 1992). There are numerous report about carry over of aflatoxin B1 from feed to milk of cows, but much less about carry over aflatoxin from feed to goat milk (Yousef & Marth, 1989).

Pasteurization, fermentation or cooling does not have changing effect on content of aflatoxin in milk because aflatoxins are very stable compounds (Alcroft & Carnaghan, 1962; Yousef & Marth, 1989; Wiseman & Marth, 1983). On the other hand processes of making cheese significantly increase content of aflatoxin, from 2.5 to 5.8 times higher, depending from the type of cheese (soft or hard).

This study was carry over on goat feed material and milk, taken from the small individual farms, in the area of north west Serbia. We investigated different feed material which was used for goat’s diet in the period from February to May 2007 and also we take from each farm one sample of milk from randomly chosen goat to analyze on content of aflatoxin M1.

Milk produced on the small farms in Serbia usually is used for making cheese or is used as row milk.

**Material and methods**

From each farm from which were taken samples of milk also were taken samples of different feed material used for feeding. Because aflatoxin B1 is not randomly distributed in feed (what is not case in milk for aflatoxin M1), usually exist small pockets with very high contamination, special notice were taken for taking samples.

Samples of feed were analyzed on aflatoxin B1, using method of extracting with organic solvent and determining with TLC (Balzer, 1978).

All 18 samples of row milk were analyzed to determine AFM1, from 18 different individual farms, from randomly picked animals on each farms (where was more than one animal). Milk was stored in freezer on -20 °C until were analyzed. All eighteen samples were taken from the February to May 2007, and didn’t pass any type of biotechnology process.

Method which was used to determine aflatoxin M1 combine clean up process with immunoaffinity columns and TLC determination (Grosso et al., 2004; Shundo L. in Sabino M 2006). The milk samples were centrifuged 15 min and the upper fat layer was discarded. The skimmed milk (100ml) was washed with distillated water (40 ml). Bound aflatoxin M1 in the immunoaffinity column was released by the elution with 2,5 ml acetonitrile-methanol (3:2; v/v) and 2,5 ml methanol, the elute was evaporated to dryness using rotary evaporator.

![Fig. 1](image-url) With red color is marked places of farm from which were taken samples of milk and feed
Occurrence of aflatoxin B1 in feed material and aflatoxin M1 in goat milk in the area of...

trate of AFM$_1$ was resuspended in 1 ml acetonitrile and again evaporated. The last concentrate was re-suspended in 200 μl toluene acetonitrile (9:1; v/v).

For processing TLC procedure was used concentrate samples from 50 and 100 μl and on TLC plate samples and working standard were applied (TLC aluminum sheets, 20x20 cm, Silica gel 60). The plates were developed in chloroform:acetone:isopropanol (87:10:3; v/v). After the plate had dried it was read under long wave (366nm) light and it was determining of concentration of AFM$_1$ by taking into account spots of samples and spots of standards.

**Investigated area**

It was eighteen samples of feed material and eighteen samples of milk, taken from eighteen different farms (from one farm it was taken one sample of milk from randomly picked goat and one samples of feed used for feeding animals). All farms where from the north west part of Serbia, from the region of Backa, Srem and Macva, and samples were taken in from the period of February 2007 to May 2007.

**Results**

**Feed**

Results of analyzed feed material are divided in two group according EU regulatory limit (limit for AFB$_1$ in feed in EU is 5 μg/kg (EFSA, 2004), while in Serbia that limit is 10 μg/kg (Sluzbeni list SFRJ 1990)).

As we can see in table number 1, ten samples (56%) from eighteen had less AFB$_1$ than it is regulator limit in EU, and eight samples (44%) had AFB$_1$ above statutory limit in EU.

**Tab. 1.** Occurrence of AFB$_1$ in feed material used for feeding goat

<table>
<thead>
<tr>
<th>Feed</th>
<th>samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 μg/kg AFB$_1$</td>
<td>10</td>
<td>56</td>
</tr>
<tr>
<td>&lt;5 μg/kg AFB$_1$</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Σ</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

**Milk**

Eighteen samples of goat milk from different individual farms were analyzed. The limit of detection was 0.0125 μg/l of aflatoxins M$_1$. All data are divided into three groups: up to 0.0125 μg/l, from 0.125 to 0.05 μg/l and values of AFM$_1$ higher from 0.05 μg/l. Summarized results are shown in table No 3.

The incidence of aflatoxin M$_1$ are shown in table number 3, where it is possible to see that 38.89% samples had more aflatoxin M$_1$ than 0.05 μg/l. According to our regulation in which is permitted limit 0.5 μg/l none samples have higher concentration from this limit.

**Tab. 3.** Result of analyzed goat milk for the presence of aflatoxin M$_1$

<table>
<thead>
<tr>
<th>Goat milk</th>
<th>&lt;0.0125 μg/l</th>
<th>0.0125-0.05 μg/l</th>
<th>&gt;0.05 μg/l</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Percent</td>
<td>33.33</td>
<td>27.78</td>
<td>38.89</td>
<td>100</td>
</tr>
</tbody>
</table>

**Graphic 1.** Illustrated different incidence of concentration aflatoxin M$_1$ in samples of goat milk
### Discussion

Eighteen analyzed samples of different feed material used for feeding goat on small individual farms in the region of north-west Serbia were analyzed on the presence of AFB\(_1\), 44% had more than it is permitted level in EU (5 µg/kg), while the rest of samples had less AFB\(_1\), than it is permitted by EU legislation. As country of Europe we should not have problem with AFs, but in some year’s storage and favored climate condition can favored growth of mold from the genus of *Aspergillus* and therefore occurrence of aflatoxins in feed and consequently occurrence of aflatoxin M\(_1\) in milk.

Eighteen analyzed samples of goat milk, taken from individual farms, incidence of occurrence aflatoxin M\(_1\) in concentration >0,0125 µg/l was 33 %, while from 0,125 to 0,05 µg/l was 28 %, and samples which exited 0,05 µg/l was 39 %, while not was samples with higher concentration from 0,5 µg/l (0,5 µg/l is regulatory limit according to Serbian standards, (Sluzbeni list SFRJ, 1990)). Weighted mean concentrations of aflatoxin M\(_1\) in all samples of milk was 0,049 µg/l.

If we see some date from the countries of Europe, it is clear that occurrence of AFs can happened, whether from imported food and feed, or from produced food in Europe. Exemplar is Italy, where in north part favored climate condition favored grow of *Aspergillus flavus*, and therefore production of aflatoxins which resulted in some samples (7,8 %) of milk were AFM\(_1\) above statutory limit (EFSA, 2004).

### Conclusions

This result showing that we should refer on need of constant monitoring feed and milk in Serbia on mycotoxins, if we wont to protect our population and to have insight of happening on our market. It can happen that we have problem on our market, but that problem can pass unnoticed.

On the basis of the weighted mean concentrations of aflatoxin M\(_1\) in milk in the regions of Serbia, assuming that daily intake of aflatoxin M\(_1\) in milk and milk products is the same or similar like in Europe, 340g per day (Henry et all, 2004) per one persona, and if we use this data to calculated average daily intake of aflatoxin M\(_1\), estimation is that daily intake of aflatoxin M\(_1\) in the analyzed region is 17ng per day, per one persona, which is about three time higher than in Europe 6,8 ng (Henry et all, 2004).

### Acknowelegment

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Summary

This result showing that we should refer on need of constant monitoring feed and milk in Serbia on mycotoxins, if we wont to protect our population and to have insight of happening on our market. It can happen that we have problem on our market, but that problem can pass unnoticed.

On the basis of the weighted mean concentrations of aflatoxin M₁ in milk in the regions of Serbia, assuming that daily intake of aflatoxin M₁ in milk and milk products is the same or similar like in Europe, 340g per day per one persona, and if we use this date to calculated average daily intake of aflatoxin M₁, estimation is that daily intake of aflatoxin M₁ in the analyzed region is 17 ng per day, per one persona, which is about three time higher than in Europe 6,8 ng.