Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure

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A B S T R A C T

The level of aflatoxin M1 (AFM1) in 50 milk samples collected from February to June 2013 from Serbian market or domestically produced was determined using simple non-specific sample preparation method based on solid phase extraction (Oasis HLB, Waters) and ultra-high performance liquid chromatography with heated electrospray ionization triple quadrupole mass spectrometry (UHPLC/ESI-MS/MS). The range of detection was between LOD and 1.44 µg/kg with mean value of 0.30 µg/kg. Thirty-eight samples (76%) exceed the maximum level of 0.05 µg/kg sets by EU. The highest level of 1.44 µg/kg was found in raw sample of domestically produced milk while the lowest one in organic produced milk. The evaluation of the exposure degree of AFM1 through the milk consumption by the average Serbian citizen was estimated at levels of 1.420, 0.769 and 0.503 ng/kg bw/day during February, April and May, respectively. Estimation of the corresponding concentration of AFB1 in feedstuffs was evaluated as 18.75 µg/kg. The calculated hazard index of 71, 3.8 and 2.5 for February, April and May, respectively, was higher than 1 indicated serious risk of AFM1 to Serbian consumers. This work presents the first insight in the occurrence of AFM1 in milk collected in Serbia as well as mycotoxin intake through milk consumption by Serbian adult population.

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1. Introduction

Animals are exposed to mycotoxins such as the aflatoxins (AFs) by consumption of feeds contaminated by mycotoxin-producing moulds during growth, harvest and/or storage. When lactating cows consume aflatoxin B1 (AFB1) contaminated feed, AFB1 is metabolized to form the monohydroxy derivative, aflatoxin M1 (AFM1), which is expressed in the cow’s milk. The AFM1 is the main hydroxylated derivative of AFB1 formed in liver by means of P450 cytochrome enzymes and secreted into milk through the mammary gland of dairy cows (Tsakiris et al., 2013). In high-yielding cows, the consumption of significantly higher amounts of concentrated feeds might result in carry-over percentages as high as 6.2% (EFSA, 2004). The AFM1 derivative can be detected in milk within 12–24 h after the first intake of AFB1, while its concentration decreases to an undetectable level 72 h after the initial intake is stopped (Tsakiris et al., 2013).

International Agency for Research on Cancer (IARC, 2002) classified AFM1 in Group 1 toxin as possibly carcinogenic for humans. The AFM1 is not destroyed by pasteurization of milk, and can thus be transferred into yoghurt, powdered milk and other milk based products (JECFA, 2001).

In fact, the contamination of milk and milk products with AFM1 may vary according to geography, environmental and climate conditions, and development level of the country (Ghazani, 2009; Prandini et al., 2009; Rahimi, Bonyadian, Rafei, & Kazemeini, 2010). The presence of AFs in feed used for the cows diet is also a very significant factor that influences AFM1 level in milk and dairy products.

Milk is considered to be a perfect natural food for consumers of all age groups due to its high nutritional value. It is high in protein and a valuable source of calcium, vitamins and antioxidants (Zeluta et al., 2010). However, milk has the greatest potential demonstrated for introducing AFM1 into human diet. The frequency of occurrence of AFM1 in commercially available milk and dairy products, the high intake of these products by human population, especially by infant and young children and its probable carcinogenic effect, led to an increased concern about the establishment of measures to control AFM1 contamination. In the light of these concerns, several countries have established regulatory limits for AFM1 in milk and derivative products, with values varying according to national legislation. According to the Food and Agriculture Organization, sixty countries have established regulatory...
limits for AFM1. The European Commission Regulation 1881/2006 sets a maximum limit of 0.05 μg/kg for AFM1 in raw milk, heat-treated milk and milk for the manufacture of milk based products (EC, 2006a). The same limit was also valid in Serbia from 2011 (Serbian regulation, 28/2011) till the end of February 2013. However, after the outbreak of milk contamination by AFM1 that occurred in Serbia in the very end of February 2013, the Serbian Government has established new maximum level of 0.5 μg/kg (Serbian regulation, 20/2013), seemed to be a practical compromise between the need to control AFM1 and the economic consequences of the setting regulatory limit. This value, although considered at the time as an interim measure to deal with the crisis, has not been updated yet.

There have been scarce data on the occurrence of AFM1 in milk produced in Western Balkan Countries. Thus, this work aims to provide the information on the occurrence of AFM1 in different types of milk collected from the main milk brands marketed in Serbia and also domestically produced during three months and to investigate if the decreasing trend is existed. According to the obtained levels of AFM1, the mean daily intake of AFM1 is estimated for Serbian adult consumer through milk consumption. In addition, AFM1 milk content is used as a biomarker of cows exposure to AFM1 through the feeds highlighting the importance of the feed-to-food chain safety.

2. Materials and methods

2.1. Reagents and chemicals

Standard stock solution of AFM1 (10 μg/ml) was purchased from Supelco Co. (Bellefonte, PA). The standard dissolved in acetonitrile were stored at 4 °C in amber glass vials, and brought to room temperature before use. Composite working standard solutions were prepared by diluting the above-mentioned stock solutions in acetonitrile and they were added in appropriate dilution to the extract of the uncontaminated sample to prepare matrix-matched calibration standards in concentration ranges that include the maximum allowable concentrations and also the expected range of mycotoxin occurrence (in accordance to the available literature data). Ultra-pure water was produced by Milli-Q purification system (Millipore, Molsheim, France). Methanol, acetonitrile and ammonium acetate (all LC—MS grade) were supplied from J.T. Baker (Deventer, The Netherlands), glacial acetic acid (p.a.) was obtained from LTG Promochem (Wesel, Germany). Oasis HLB (200 mg, 6 cc) cartridges were supplied from Waters (Milford, MA, USA).

2.2. Collection of samples

The collected milk samples were from the main Serbian dairy plants (1–V) with the highest capacities for the milk production (from 100,000 to 500,000 L per day) that account for 50–80% of the total raw (fresh) milk redemption in Serbia (the exact market shares of the dairy plants in the total Serbian milk production are protected data, Commission for Competition Protection of Republic of Serbia, 2012).

Forty two samples of different types of commercial milk (taking into consideration the percentage of milk fat and the milk processing, e.g. pasteurized and sterilized milk) produced in Serbia or Bosnia and Herzegovina were collected from markets in the city of Novi Sad (northern part of Serbia) in February, April and May 2013. The commercial packs from 0.25 L to 1 L were collected. The majority of samples were in a Tetrapak® type packages while the rest were bottled in plastic bottles. Six additional samples of raw milk produced in households (small private farms) in three northern counties of Serbia (Srem, Bačka, and Banat) were collected. Two additional samples of raw milk were taken from small private farm located in the south eastern mountain region of Serbia, where different feed could be assumed to be used for cows in comparison to the one used in the northern plains regions (Srem, Bačka, and Banat). It should be emphasized that samples of raw milk from small private farms were obtained on voluntary basis from the farm owners, since such samples are not commercially available on the market. The samples were stored in their original packs at 4–5 °C until analysis was carried out.

2.3. Sample preparation

The sample preparation procedure was based on the modified method previously explained by Wang et al. (2012). Samples of milk were prepared using Oasis HLB cartridges which are non-specific columns for mycotoxins and which are cheaper than immunoaffinity columns. However, for the analysis of AFM1 in milk the preferred option has been the cleanup procedure of the extracts based primarily on rather expensive immunoaffinity column-based preparatory methods, followed by the high performance liquid chromatography with fluorescence detection (HPLC-FLD) (Iqbal & Asi, 2013) or UHPLC—MS/MS (Beltrán et al., 2011).

Briefly, milk was weighed (20 g) into 50 ml centrifuge tube. After centrifugation for 10 min (8000 rpm), fat layer was removed and the supernatant was collected. Supernatant solution (10 ml) was applied to an Oasis HLB cartridge which had previously been conditioned with 5 ml acetonitrile and 5 ml water, successively. The column was washed with 5 ml 20% acetonitrile aqueous solution. The AFM1 was eluted with 5 ml acetonitrile and the eluate was collected and evaporated to dryness using gentle stream of nitrogen. The residue was reconstituted with 1 ml of 20% aqueous acetonitrile and the obtained solution was passed through the 0.2 μm nylon syringe filter.

2.4. Instrumental conditions

Ultra-high performance liquid chromatography (UHPLC) performed by Accela™ (Thermo Fisher Scientific, USA) was used for separation of sample components. Hypersil GOLD™, 50 × 2.1 mm i.d., 1.9 μm column (Thermo Fisher Scientific, USA) was used with a flow rate of 0.5 ml/min, and the column temperature was maintained at 30 °C. The injection volume was 10 μl. The mobile phase consisted of eluent A containing water/acetic acid (99:1, v/v), and eluent B consisting of methanol/acetic acid (99:1, v/v). Both eluents contained 5 mM ammonium acetate. The gradient program started with 95% A and 5% B and was kept until 0.5 min, afterward a linear gradient was applied, reaching 95% B after 3.04 min (holding time 2.1 min) and then switch back (6.20 min) to 95%A (holding time 1.80 min), which was maintained till the end of the run at 8 min. For analyte detection, triple quadrupole mass spectrometer (MS/MS) TSQ Vantage (Thermo Fisher Scientific, USA) equipped with heated-electrospray ionization probe (HESI-II, Thermo Scientific, USA) was used. Parameters of the ion source were as follows: spray voltage – 3.4 kV, vaporizer temperature – 350 °C, sheath gas pressure – 40 arbitrary units, auxiliary gas pressure – 10 arbitrary units, and capillary temperature – 270 °C.

Acquisition parameters of the mass spectrometer were optimized by direct continuous pump infusion of 5 μg/ml standard solutions of AFM1 dissolved in initial mobile phase into the mass spectrometer using a syringe pump at a flow rate of 10 μl/min. Data acquisition was performed initially in full scan to determine an abundant precursor ion. Next, the MS/MS fragmentation conditions were investigated and collision energies and S-lens voltages were optimized for AFM1 and transition of this toxin. UHPLC/HESI-MS/MS parameters of AFM1 separation and identification under optimized
conditions on the Accela-TSQ Vantage system were: retention time − 2.86 min, dwell time − 0.1 s, precursor ion \((m/z) - 329\) \([M + H]^{-}\). Fragmentation reaction was done in selected reaction monitoring mode (SRM) by choosing the optimum voltage of collision energies for selected compound. Two product ions were measured for precursor ion: one was used as the quantifier ion (273.1) and the other was used as the qualifier ion (259.1). Collision-induced dissociation energy for quantifier ion was 25 eV, while for qualifier ion it was 23 eV. In SRM mode, a mass resolution of 0.7 Da full width at half maximum (FWHM) was set on the first (Q1) and the third (Q3) quadrupole and a scan width of 0.5 m/z were used. Instrument control and data collection were handled by computer equipped with Xcalibur 2.1.0 (Thermo Fisher Scientific, USA).

2.5. Validation of the method

The developed method was validated by “in-house” quality control procedure. Parameters taking into account were: instrumental linearity, limits of detection (LOD) and quantification (LOQ), recovery and precision (expressed as relative standard deviation, RSD).

The AFM1 was quantified by external matrix-matched calibration procedure. Calibration solutions for matrix-matched calibration curve were prepared in uncontaminated milk extract. Linearity of the method was estimated by analysis of six calibration solutions (solvent- and matrix-matched standards) in triplicate in the range from 0.025 \(\mu g/kg\) to 2 \(\mu g/kg\). LOD and LOQ were calculated analyzing matrix matched standards at the lowest calibration level, and they were determined as the lowest concentration of the analytes that produce chromatographic peak at S/N of 3 and 10, respectively.

Validation of the analytical method was carried out by determination of recoveries (“in-house”) of uncontaminated milk sample spiked at levels of 0.05 \(\mu\)g/kg and 0.5 \(\mu\)g/kg for AFM1. Recovery experiments were performed in triplicates for both levels. Spiked samples were left overnight at room temperature to allow solvent evaporation and equilibration between analyte and matrix.

The repeatability of the method was determined as relative standard deviation (RSD, in %) of AFM1 content in three fortified samples of milk.

In order to evaluate matrix effects, the signal suppression/enhancement (SSE) for AFM1 in milk sample was calculated, defined as the percentage of the matrix-matched calibration slope divided by the slope of the standard calibration in solvent:

\[
SSE(\%) = 100 \times \frac{\text{matched slope of matrix}}{\text{based calibration curve}}
\]

In the absence of matrix effects, the slopes in two calibration plots correspond each other within the experimental error deviation. A lower slope in the calibration plot obtained for the matrix-matched standards (SSE < 100%) indicates the suppression of the analyte signal, while a greater slope (SSE > 100%) indicates signal enhancement.

All samples were analyzed in triplicates. Blank samples were included in every batch of samples to check for possible contamination.

2.6. Intake calculation

Calculation of the exposure of Serbian population by milk consumption was done by combining data on the average daily consumption of the milk with the average mycotoxin concentration found here, as follows:

\[
\text{Estimate of mycotoxin intake (ng/kg bw/day)} = \frac{[\text{toxin}] \times [\text{milk consumption}]}{[\text{bw}]}
\]

where [toxin] is the average concentration of mycotoxin in (ng/kg) detected in milk adjusted for recovery, [milk consumption] is the amount of milk (kg) consumed per person per day, and [bw] is the body weight (kg).

Milk consumption amount used for calculation of the intakes was obtained according to the Serbian market basket (Statistical Office of the Republic of Serbia, 2011). According to the Serbian market basket (Statistical Office of the Republic of Serbia, 2011) individual average consumption of milk is 177.5 g/day. The average body weight (bw) of 60 kg for adults was used for calculating daily intakes per kg bw.

The estimation of hazard index (HI) is based on proposal of Kuiper-Goodman (1990) who have estimated tolerable daily intake (TDI) for AFM1 by dividing the TD50, (threshold dose per body weight) with uncertainty factor of 5000. The proposed value of TDI is 0.2 ng/kg bw/day is equivalent to a risk level of 1:100,000. According to the HI the risk assessment scenario could be evaluated. The HI higher than 1 indicates risk to consumers.

2.7. Calculation of extrapolated values of AFM1 concentration in cattle feeds

The values of AFM1 in cattle feeds were extrapolated from back calculation of the values of AFM1 obtained from analysis of milk samples. The calculation was performed considering that 1.6% of ingested AFM1 is converted to AFM1 by lactating cattle (Price, Paulson, Lough, Ginnig, & Kurtz, 1985) as estimated by following formula:

\[
\text{AFB1 (\mu g/kg)} = \frac{\text{AFM1 (ng/kg)} \times 100}{1.6 \times 1000}
\]

3. Results and discussion

3.1. Method validation parameters

Method performance characteristics studied were linearity, LOD and LOQ, recovery and repeatability of the method.

The linearity of the calibration graphs obtained for the matrix-matched standards was evaluated by calculation of the squared correlation coefficient \(R^2\). \(R^2\) for matrix-matched calibration curve prepared in each sampling period was above 0.990 showing excellent linearity. In case of the solvent based calibrants \(R^2\) of the calibration curve was >0.990.

LOD of AFM1 (0.0002 \(\mu g/kg\)) was lower than the maximum residue limits established by European Union (EC, 2006a), indicating the suitability of the proposed method for the determination of trace concentration of selected compound. LOQ of AFM1 was 0.0007 \(\mu g/kg\).

Recovery of method was evaluated by analyzing fortified blank samples in triplicates at the level (0.05 \(\mu g/kg\)) corresponding to the maximum value allowed by the European Commission and as well as at the level of 0.5 \(\mu g/kg\) sets by the new Serbian legislation (20/2013). The mean recovery value for spiking level of 0.05 \(\mu g/kg\) was 69%, while for 0.5 \(\mu g/kg\) the obtained recovery was 71%, being in compliance with Commission Regulation (EC, 2006b).
uncontaminated milk sample spiked at levels of 0.05 \( \mu \text{g/kg} \) (1b) and of 0.5 \( \mu \text{g/kg} \) (1c).

For AFM1 considered, good repeatability was obtained for both spiking levels, since the RSD for the added levels was below 11% being in accordance with request of Commission Regulation (EC, 2006b).

In order to evaluate the occurrence of SSE in the used detection system, responses of matrix-matched standards were compared with responses of the solvent-based ones. The SSE was calculated by Eq. (1), expressing the ratio of matrix-matched calibration slope to solvent calibration slope in the whole identical calibration range. Calibration curve in solvent was obtained for the calibration standards prepared in the mobile phase; for matrix-matched calibration curve, the calibration standards were prepared in blank milk extracts. Investigated matrix induced ion suppression occurred for AFM1, since SSE value was lower than 100% (an SSE of 100% indicated that matrix have no effect on the MS signal). Calculated SSE value for AFM1 was 86%.

### 3.2. Occurrence of AFM1 in milk

Occurrence of AFM1 in real samples of milk collected in three periods (February, April and May 2013) was presented in Table 1. The presented results were corrected for “in-house” recovery. The occurrence of AFM1 indicated that the level of this toxin was the highest during February followed by April and May; the average value of AFM1 in February samples was 0.48 \( \mu \text{g/kg} \), while in April and May, it was 0.26 and 0.17 \( \mu \text{g/kg} \), respectively. The contamination levels of the AFM1 toxin in milk samples collected in February ranged from <LOD to 1.44 \( \mu \text{g/kg} \), while the concentration range of studied toxin in milk samples collected in April was from <LOD to 0.72 \( \mu \text{g/kg} \) (Table 1). Remarkably, lower concentration range of AFM1 (<LOD–0.49 \( \mu \text{g/kg} \)) in milk samples collected in May was obtained (Table 1). In February, 80% of the analyzed samples were significantly contaminated with AFM1 toxin with levels above 0.05 \( \mu \text{g/kg} \) sets as the maximum allowable concentration by EU (Table 1). Almost the same percentage of the positive samples with AFM1 above EU limit was obtained for samples taken in April.

![Fig. 1. UHPLC–MS/MS chromatograms for AFM1 corresponding to: a) uncontaminated sample (<LOD), b) uncontaminated milk sample spiked at level of 0.05 \( \mu \text{g/kg} \), c) uncontaminated milk sample spiked at level of 0.5 \( \mu \text{g/kg} \) d) the real sample with the lowest level of AFM1 (0.01 \( \mu \text{g/kg} \)) and e) the real sample with the highest level of AFM1 (1.44 \( \mu \text{g/kg} \)).](image-url)
Frequency of occurrence of AFM1 (69%) in samples collected in May was lower than for the investigated ones in February and April. In total, only 12 out of 50 investigated milk samples were not contaminated taking into consideration level of 0.05 μg/kg sets by EU regulation (EC, 2006a). Considering the new value sets for AFM1 by the Serbian regulation in February 2013 (Serbian regulation, 20/2013), only one of the investigated milk samples produced in Serbia was not above the Serbian valid limit 0.05 μg/kg (see samples of pasteurized milk with 2.8% of fat from dairy plant I; Table 1). The lowest levels of AFM1 were determined in samples of organic milk produced at the dairy plant I. It is worth to note that organic milk from this dairy plant is the only commercially available organic milk in Serbia. Furthermore, very low level of AFM1 was found in milk collected from the private farm from the mountain region of Serbia during February and April (0.01 μg/kg, Table 1). Comparison of the data obtained for milk samples taken from private farms from northern part of the county i.e. of Banat, Backa and Srem, with those from the farm in the mountain region indicated that different feed provided to cows in these distant regions was probably the cause of different levels of AFM1.

According to the study carried out in Brazil (Iha, Barbosa, Okada, & Truckess, 2013) AFM1 was detected in 46% of ultra high-temperature milk samples in the range from 0.008 to 0.215 μg/kg, while in 86% of pasteurized milk samples concentration range of this toxins was from 0.009 to 0.437 μg/kg. A survey done by Zheng et al. (2013) revealed that 96.2% of analyzed samples were positive for AFM1 with concentration range from 0.023 to 1.154 μg/L Picinini et al. (2013), in Brazil, analyzed 129 raw milk samples, and the levels of the AFM1 in analyzed samples ranged from 0.0002 to 0.1057 μg/L. In Portugal, Duarte et al. (2013), analyzed AFM1 in 40 half-skimmed milk samples and concentration of the investigated toxin was at levels ranging from 0.0069 to 0.0697 μg/L. Iqbal and Asi (2013) analyzed 107 milk samples in Pakistan, of which 76 (71%) were positive with AFM1 at levels from 0.004 to 0.845 μg/L. In Greece, Tsakiris et al. (2013) analyzed 196 milk samples and detected AFM1 in 46.5% of the samples with concentration from 0.005 to 0.01 μg/L being lower than the EU limit.

### Table 1

<table>
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<tr>
<th>Sample origin</th>
<th>% Of milk fat</th>
<th>Type of milk</th>
<th>February, 2013</th>
<th>April, 2013</th>
<th>May, 2013</th>
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<tr>
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<td>Sterilized</td>
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<td>0.43</td>
<td>0.29</td>
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<td></td>
<td></td>
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<tr>
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<td>Sterilized</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
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<tr>
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<td>Sterilized</td>
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<td>0.05</td>
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<td>0.03</td>
<td>0.06</td>
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<td>Sterilized</td>
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<td>1.44</td>
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<td>Settlement in the Srem county, Serbia</td>
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<td>0.5 μg/kg</td>
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<tr>
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<td>0.05 μg/kg</td>
<td>0.05 μg/kg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Dairy plant of Bosnia and Herzegovina.
b The results presented in the same row obtained for the same dairy plant during different sampling periods.
c Only one of the investigated milk samples produced in Serbia was not above the Serbian valid limit.
d Pasteurized or raw (boiled) milk.
e Organic milk.
f In May, this samples were not available on the Serbian market.
In this study, AFM1 levels in milk appeared to be higher than those found in some other studies published this year. However, comparison among the countries is difficult due to different analytical procedures applied, climatic period, storage conditions, the sampling period, etc. Knowing that the occurrence of AFM1 in milk originates from the presence of AFB1 in feed (Tsakiris et al., 2013), differences between AFM1 in samples from various studies may be attributed, among others, to a different origin of feed and AFB1 contamination of feed.

3.3. Estimated daily intake of AFM1 in human consumers through milk consumption

The average daily intake of AFM1 was estimated using the obtained average level of AFM1 in milk samples and milk consumption rate by the average Serbian adult consumers. The estimated intakes of AFM1 for the general Serbian adults were 1.420, 0.769 and 0.503 ng/kg bw/day during February, April and May, respectively, through consumed milk. The reported values could be underestimated due to fact that the estimated values are based only on milk consumption and do not include the other dairy foods such as cheese, yogurt, ice cream, etc. The estimated intakes of AFM1 in this study are higher than the one for the adult population of Spain at 0.305 ng/kg bw/day (Cano-Sancho, Marin, Ramos, Peris-Vicente, & Sanchis, 2010), but lower than the one for Morocco reported to be 3.26 ng/kg bw/day (Zinedine et al., 2007). At the international level, on the bases of the mean concentration of AFM1 in milk and the milk consumption (Zinedine et al., 2007). The estimated intakes of AFM1 in human consumers through consumed milk does not exist for children and infants, thus, the estimation of daily intakes for these groups was not possible.

Although AFs are considered to be genotoxic carcinogens, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) of European Community did not establish tolerable daily intake (TDI) for AFM1. They concluded that daily exposure, even below 1 ng/kg bw, contributed to the risk of liver cancer. In the light of these concerns, international expert committees (JECFA, 2001) recommended that concentration of AFs in food should be as low as reasonably achievable. Additionally, taking into consideration the TDI proposed by Kuiper-Goodman (1990) hazard index (HI) is also calculated to assess the risk assessment scenario. High exposure assessment scenario for AFM1 according to the HI obtained (7.1, 3.8 and 2.5 for February, April and May, respectively) were estimated for the average Serbian adult consumers. The determined values of HI for AFM1 through the consumption of milk could be order as follows: 7.1 > 3.8 > 2.5, from February to May. HI calculated for each of investigated periods was higher than 1 indicated serious risk of AFM1 to Serbian consumers, but also decreasing trend of daily intake and HI.

3.4. Extrapolated intake of AFB1 in feed

It is known that contamination of AFM1 in milk is a result of exposure of AFB1 to dairy cattle through feedstuffs. Based on AFM1 contamination of milk, the calculated average concentration of AFB1 (according to Price et al. (1985)) in feeds consumed by the producing cows was 18.75 µg/kg, which is 3.8 times higher than the maximum allowed level in feeds for dairy cattle set by the European Directives (Directive 2002/32/EC (EC, 2002) and amending Directive 2003/100/EC (EC, 2003) to be 5 µg/kg. Additionally, levels of AFB1 in feeds consumed by the producing cows during February, April and May were 30, 16.25 and 10.63 µg/kg, respectively. However, this estimation is done considering a carry-over rate of 1.6% AFB1 as it is suggested (Price et al., 1985). However, taking into consideration reported (EFSA, 2004) carry-over rates that range from 2% (assumed average level) to 6% (reported level for high yielding cows) the extrapolated intake of AFB1 in feed is underestimated indicating that control measures need to be implemented in the initial food chain highlighting the monitoring of dairy products as well as feed provided to animals.

4. Conclusion

The present study is a first comprehensive survey investigating the occurrence of AFM1 in different types of milk available in Serbia.

The occurrence of AFM1 was detected at different levels in 50 investigated milk samples from the Serbian market or domestically produced, collected in period from February to June this year. The contamination levels of 38 samples (76%) were above the maximum allowed limit sets by European legislation. The calculated hazard index for February, April and May being almost 7, 4 and 3 times higher than 1 indicated serious risk of AFM1 to Serbian consumers. Therefore, the high level of AFM1 in investigated milk confirmed that constant monitoring throughout the milk production chain is necessary in order to minimize health risks related to the presence of this toxin in milk. Reduction of the levels of AFM1 in milk can be achieved by the implementation of good agricultural and storage practices to control the risk of toxicogenic fungi and AFs contamination along the feed supply chain and also by setting stringent regulatory limits for AFs in feed and milk in Serbia.

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