Short communication: Investigation of aflatoxin M₁ levels in infant follow-on milks and infant formulas sold in the markets of Ankara, Turkey

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ABSTRACT

Aflatoxins are fungal toxins known to be carcinogenic and are classified as food contaminants. This study was performed to investigate aflatoxin (AF) M₁ levels in baby foods sold in Ankara (Turkey) and to evaluate the obtained results according to the Turkish Food Codex (TFC). For this purpose, a total of 84 baby food samples (50 follow-on milks and 34 infant formulas) were obtained from different markets in Ankara and the presence of AFM₁ in the samples was analyzed by ELISA. In 32 (38.1%) of 84 infant food samples, the presence of AFM₁ was detected in concentrations ranging between 0.0055 and 0.0201 µg/kg. The mean level (±standard error) of AFM₁ was found to be 0.0089 ± 0.0006 µg/kg in positive infant follow-on milks. Aflatoxin M₁ was detected in only 1 infant formula sample (2.94%) at a concentration of 0.0061 µg/kg. The extrapolated levels of AFB₁ contamination in feedstuffs were calculated based on levels of AFM₁ in baby food samples. The data estimating AFB₁ contamination in dairy cattle feedstuff indicate that contamination may range from 0.3410 to 1.2580 µg/kg, with the mean level (±standard error) being 0.5499 ± 0.0385 µg/kg, which is lower than the level set by the TFC and European Union regulations (5 µg/kg). According to the obtained results, the levels of AFM₁ in analyzed samples were within the allowed limit (0.025 µg/kg) set in the TFC. Low levels of AFM₁ in infant follow-on milks and infant formula samples obtained during the study do not pose a health risk to infants.

Key words: aflatoxin M₁, aflatoxin B₁, infant food, ELISA

Short Communication

Aflatoxin M₁ (AFM₁) is a hydroxylated metabolite of aflatoxin B₁ (AFB₁), found in the milk of dairy cattle fed AFB₁-contaminated feedstuff (Sugiyama et al., 2008). When contaminated feedstuffs are consumed by

lactating animals, AFB₁ is absorbed in the gastrointestinal tract and is biotransformed to AFM₁ by hepatic microsomal cytochrome oxidase P450 in the liver and it is excreted in the milk (de Oliveira et al., 2013). Aflatoxin M₁ is stable at high temperatures and cannot be removed from milk by heat processing; that is, pasteurization and ultra-high temperature (Duarte et al., 2013; Iqbal et al., 2013). Therefore, AFM₁ can be transferred to dairy products such as milk powder, cheese, butter, and yogurt (Kabak, 2012; Duarte et al., 2013). Aflatoxin M₁ can also be found in infant foods (Kabak, 2012). For this reason, even very low levels of AFM₁ are very important in terms of infant and child health (Duarte et al., 2013). Therefore, the analysis of the AFM₁ levels in baby and infant foods is very important.

Aflatoxin M₁ was reclassified as a group-1 carcinogen in 2002 by the International Agency for Research on Cancer (IARC, 2002). According to the Turkish Food Codex (TFC), the level of AFM₁ in infant follow-on milks and infant formulas should not exceed 0.025 µg/kg (TFC, 2011). Aflatoxin M₁ residues are permitted in infant foods up to 25 ng/kg in the European Union (EC, 2010).

Our aim was to determine the presence and levels of AFM₁ in baby food samples in Ankara (Turkey) markets and to evaluate whether AFM₁ levels were within the Turkish Food Codex values or not. Also, extrapolated levels of AFB₁ in feedstuffs were calculated based on levels of AFM₁ in infant food samples.

A total of 50 infant follow-on milks of 4 different companies (A, B, C, and D) and 34 infant formula samples of 3 different companies (E, F, and G), were collected from markets in Ankara, Turkey. Infant food samples that had different serial numbers were used. These samples were collected before their expiration date and stored at room temperature until they were opened.

The levels of AFM₁ were determined with an ELISA, using the Ridascreen AFM₁/ELISA kit (R-Biopharm AG, Darmstadt, Germany; R-Biopharm AG, 2012). Samples were prepared according to the instructions of the Ridascreen kit procedure. One hundred microliters of antibody was added to the 96 wells, mixed gently, and incubated for 15 min at room temperature. One

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Figure 1. Aflatoxin M₁ levels (mean ± SE) of infant follow-on milks.

hundred microliters of the standard solution and the prepared samples was added to wells, mixed, and incubated for 30 min at room temperature in the dark. One hundred microliters of enzyme conjugate was added to the wells, mixed gently, and incubated for 15 min at room temperature in the dark. One hundred microliters of the substrate/chromogen was added to the wells and incubated at room temperature for 15 min in the dark. After each incubation process, washing procedures were performed. The content was poured from the wells and washed twice with the washing buffer. One hundred microliters of the stop solution was added to each well and the absorbance was measured at 450 nm in an ELISA plate reader (VersaMax Tunable; Molecular Devices LLC, Sunnyvale, CA). Aflatoxin M₁ levels were calculated using the guidelines of the Ridascreen kit (R-Biopharm AG, 2012). The detection limit was reported as 5 ng/L in the test kit.

When contaminated feedstuffs with AFB₁ are consumed by dairy animals, 1.6% of the AFB₁ is expected to be converted to AFM₁. Hence, AFB₁ contamination of feedstuffs is calculated by the back-calculation formula $AFB_1 (\mu\text{g/kg}) = [AFM_1 (\text{ng/kg}) \times 100] / (1.6 \times 1,000)$ of AFM₁ according to Rastogi et al. (2004). A nonparametric test and Kruskal-Wallis test were conducted for statistical comparison (Rosner, 2006). The test absorbances were entered manually from the plate reader to Ridasoft Win software (R-Biopharm AG) to plot standard curves and quantify the test results. The analysis was repeated 2 times for each sample.

Graphics of AFM₁ and extrapolated AFB₁ levels (mean ± SE) of infant follow-on milks and infant formulas with error bars are given in Figures 1 and 2.

The results obtained show that 32 of 84 (38.1%) examined samples were found to contain AFM₁. Thirty-one (62.0%) of the infant follow-on milk samples and 1 (2.94%) of the infant formula samples were positive for AFM₁. The mean level (±SE) of AFM₁ was found to be $0.0089 \pm 0.0006 \mu\text{g/kg}$ in infant follow-on milk samples. However, AFM₁ was found to be $0.0061 \mu\text{g/kg}$ in only 1 out of 34 infant formula samples. This study indicates that some infant foods sold in Ankara contained AFM₁, although these levels are within allowed limits in TFC ($0.025 \mu\text{g/kg}$).

Aflatoxin M₁ in milk and dairy products shows the exposure of contaminated feedstuffs with AFB₁. Extrapolated AFB₁ levels in dairy cattle feedstuffs can be calculated based on AFM₁ levels in infant milk products according to Rastogi et al. (2004). In the present study, extrapolated mean levels (±SE) of AFB₁ were calculated to be $0.5499 \pm 0.0385 \mu\text{g/kg}$, based on AFM₁ contamination in infant foods. Aflatoxin B₁ levels were below the accepted levels ($5 \mu\text{g/kg}$) established for feedstuffs by the TFC and European Union regulations (EC, 2002; TFC, 2005).

Limited studies on AFM₁ in infant foods have been performed by several authors in Turkey. The present study indicated that the mean level of AFM₁ was $0.0088 \pm 0.0035 \mu\text{g/kg}$ in 38.1% of 84 infant food samples. Baydar et al. (2007) reported that the mean level of AFM₁ was $0.07 \pm 0.05 \mu\text{g/L}$ in 36.5% of 63 infant formulas, follow-on formulas, and baby food samples in Ankara. Kabak (2012) determined that 5 (8%) of 62 formula samples were positive for AFM₁, in the range $0.016 \mu\text{g/kg}$ to $0.022 \mu\text{g/kg}$. These AFM₁ incidences

Figure 2. Extrapolated aflatoxin B₁ levels (mean ± SE) in dairy cattle feedstuffs based on infant follow-on milks.

in infant food samples were lower compared with the value reported in present study.

To consider the hazard of AFM₁ occurrence in infant foods, various studies have been carried out for the food hygiene in different countries (Rastogi et al., 2004; Oveisi et al., 2007; Alvito et al., 2010; Gómez-Arranz and Navarro Blasco, 2010; Meucci et al., 2010; El-Tras et al., 2011). Rastogi et al. (2004), analyzed AFM₁ in 87 samples of infant food in India and 87.3% of them were positive for AFM₁ residues. According to the extrapolation of AFB₁ from AFM₁ contamination in infant products, AFB₁ contamination levels varied from 1.4 to 63.3 µg/kg, with a mean level of 18 µg/kg. Oveisi et al. (2007) determined that mean AFM₁ level was 7.3 ng/kg in 96.6% of infant formulas in Iran. El-Tras et al. (2011) showed that the AFM₁ mean level was 9.796 ± 1.036 ng/L in 43.2% (125) of the positive infant formula milk powders in Egypt. Aflatoxin M₁ incidences of infant foods were higher in studies carried out by Rastogi et al. (2004), Oveisi et al. (2007), and El-Tras et al. (2011).

Literature is also available about the occurrence of AFM₁ in infant food samples, indicating lower levels of contamination. Gómez-Arranz and Navarro-Blasco (2010) reported that the mean AFM₁ level was 3.1 ng/kg in 37.7% of the 69 different infant formulas in Spain. In another study carried out by Alvito et al. (2010), AFM₁ was found in 4 of 27 baby food samples between 0.017 and 0.041 µg/kg in Portugal. Meucci et al. (2010) analyzed AFM₁ in 185 different infant foods and only 2 samples were positive for AFM₁, in a range from 11.8 to 15.3 ng/L in Italy.

In previous national and international studies, it was observed that variability existed in the AFM₁ levels of infant foods. Therefore, many factors, including contamination of animal feedstuffs and seasonal differences, can affect the occurrence of AFM₁ (Afshar et al., 2013). In addition, AFM₁ contamination levels of milk affect several factors, such as animal breeding, lactation period, and presence of mastitis (Anfossi et al., 2011).

Aflatoxin M₁ is the most widely distributed mycotoxin in milk-based foodstuffs and has potential carcinogenic effects. Therefore, it is necessary to monitor milk and milk products for contamination levels of AFM₁. The analysis of AFM₁ in risky foods is very important for human health. According to AFM₁ limitations in infant foods by the TFC, low levels of AFM₁ in infant follow-on milks and infant formula do not pose a health risk to the consumer. These results are valid for the obtained samples during our study and the data may change over time. For this reason, monitoring strategies should be considered for preventing contamination with AFM₁ in food processing.

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