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Investigation of Aflatoxin M1 Residue in Raw Cow Milk Samples in Burdur

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ABSTRACT

This study was conducted to research the aflatoxin M_1 (AFM_1) level in food due to the economic losses and public health concerns resulting from its presence. A total of 82 raw cow milk samples were randomly obtained from dairy farms in Burdur and they were examined in terms of AFM_1 using Enzyme-Linked Immunosorbent Assay (ELISA) method. It was found that the AFM_1 level was between 5.06 and 50.63 ng kg^{-1} in 48 (58.5%) of 82 raw cow milk samples analyzed and the average contamination rate was 15.53 ±1.49 ng kg^{-1}. In 1 (1.2%) of the milk samples, AFM_1 level was found to be over the legal limits specified by Turkish Food Codex and European Union's Regulation. As a result, the AFM_1 level determined in the raw milk samples was below the maximum residue limits and was suitable for human consumption. In addition, the estimated daily intake (EDI) of AFM_1 was determined for the adult consumer in Türkiye. The average EDI (0.19 ng kg^{-1} body weight day^{-1}) of the adult consumer was found to be close to the proposed value of tolerable daily intake (0.2 ng kg^{-1} body weight day^{-1}) for AFM_1. However, it is recommended to repeat the studies on this subject within a regular program and inform both the producers and the consumers about the issue.

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ÖZET

Bu çalışma, gıdalarda aflatoksin bulunmasından kaynaklanan ekonomik kayıplar ve halk sağlığı endişeleri nedeniyle aflatoksin M_1 (AFM_1) düzeyini belirlemek amacıyla yapılmıştır. Burdur ilindeki çiftliklerden toplam 82 çiğ inek sütü örneği rastgele alındı ve Enzyme-Linked Immunosorbent Assay (ELISA) yöntemi kullanılarak AFM_1 açısından analiz edildi. İncelenen 82 çiğ inek sütü örneğinin 48'inde (%58,5) AFM_1 seviyesinin 5,06 ila 50,63 ng kg^{-1} arasında ve ortalama kontaminasyon oranının 15,53 ±1,49 ng kg^{-1} olduğu tespit edildi. Süt örneklerinin 1'inde (%1,2) AFM_1 düzeyi Türk Gıda Kodeksi ve AB Yönetmeliği'nde belirtilen yasal sınırların üzerinde olduğu belirlendi. Sonuç olarak çiğ süt örneklerinde belirlenen AFM_1 seviyesi maksimum kalıntı limitlerinin altında ve insan tüketimine uygun bulunmuştur. Ayrıca Türkiye'deki yetişkin tüketici için AFM_1'in tahmini günlük alım miktarı (EDI) belirlenmiştir. Yetişkin tüketicinin ortalama EDI'si (0,19 ng kg^{-1} vücut ağırlığı gün^{-1}), önerilen günlük 0,2 ng kg^{-1} vücut ağırlığı gün^{-1} AFM_1 alımına yakın bulunmuştur. Ancak bu konudaki çalışmaların düzenli bir program dahilinde tekrarlanması ve hem üreticilerin hem de tüketicilerin konu hakkında bilgilendirilmesi önerilmektedir.

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INTRODUCTION

Aflatoxins (AF) are a group of mycotoxins produced by the fungi species of *Aspergillus* genus, especially *A. flavus, A. parasiticus* and *A. nominus*, as secondary metabolites. The optimum growth temperature of *Aspergillus* spp. is 25ºC and the minimum water activity is 0.75. The fungi start to produce secondary metabolites at 10-12ºC. However, the most toxic ones are produced at 25°C with water activity of 0.95 (Lizárraga-Paulín et al., 2011; Kagera et al., 2018). The well-known aflatoxin species are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Kagera et al., 2018). Aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are the hydroxylated metabolites of aflatoxin B_1 and B_2 and they are found in milk of the animals fed with mouldy forage (Creppy, 2002; Yitbarek and Tamir, 2013). AFM_1 amount in milk depends on the AFB_1 concentration in the contaminated forage. Animals fed with feed containing AFB_1, these toxins are metabolized in the liver and secreted to milk as AFM_1. This is the only way to convert AFB_1 into AFM_1 (Creppy, 2002; Hassan and Kassaify, 2014). Roughly 0.3-6.2% of AFB_1 in animal feed is converted to AFM_1 in milk (Creppy, 2002; Karakaya and Atasever, 2010).

Milk and its products are a food with high nutritional value for all people, especially babies and children (Tekinsen and Ucar, 2008; Shundo et al., 2009; Yitbarek and Tamir, 2013; Kamkar et al., 2014). In addition to being consumed in liquid form, milk is used in the production of baby foods, dairy products and milk desserts and is widely consumed by people of all age groups (Tekinsen and Ucar, 2008; Kamkar et al., 2014).

Aflatoxins pass through the human placenta and they cause growth disorders in the young children exposed to aflatoxin (IARC, 2002). Especially infants are at a higher risk compared to adults due to their low body weight, high metabolic rates, insufficient detoxification, and underdevelopment of their organs and tissues (Kamkar et al., 2014). It has been reported that aflatoxins cause renal damage, cirrhosis, hepatitis, hepatocellular carcinoma, chronic gastritis, and Reye's syndrome (Henry et al., 2001; Lizárraga-Paulín et al., 2011; Li et al., 2018). AFB_1 and AFM_1 have genotoxic activity due to its potential accumulation and as it is linked to DNA (Shibahara et al., 1995). Aflatoxins also cause a decrease in milk production. The fact that AFM_1 exists in milk and dairy products is a serious food hygiene problem (Yitbarek and Tamir, 2013). AFB_1 and AFM_1 are described as carcinogenic to humans (Group 1) (IARC, 2002). Due to high toxicity and health concerns, many countries have determined maximum residue limits for AFM_1 to prevent or reduce aflatoxin risks. According to Turkish Food Codex (TFC, 2011) and European Union's Regulation (EC, 2010), the maximum AFM_1 level in milk to be used in the production of raw milk, heat-treated milk and milk-based products must be 50 ng kg^{-1} or less. According to FDA standards, the AFM_1 level in milk in the United States must be 500 ng kg^{-1} or below (FDA, 2005). For this reason, it is necessary to define the AFM_1 levels in milk and its products for the purpose of protection the health of consumers in various age groups from the possible risks (Tekinsen and Ucar, 2008; Yitbarek and Tamir, 2013; Kamkar et al., 2014).

It has been statemented that AFM_1 in milk and its products has resistance against the pasteurization, boiling, sterilization, processing at ultra-high temperature, cooling, and freezing conditions (Galvano et al., 1996; Henry et al., 2001; Awasthi et al., 2012; Iha et al., 2013; Hassan and Kassaify, 2014). A study on AFM_1 concentration changes in white cheese, it was determined that there was no important alteration in AFM_1 concentration even after a 3-month storage (Deveci, 2016). It has been reported that the AFM_1 content of milk and dairy products is not affected during cool and frozen storage. If aflatoxin-contaminated milk is utilized in the making of dairy products, the chances of AFM_1 reaching consumers significantly increase (Wiseman and Marth, 1983; Iqbal et al., 2015).

ELISA is a laboratory screening method that is preferred in food analysis as it has advanced qualifications such as high sensitivity, ease of use, rapid, low cost, and on-site monitoring (Var and Kabak, 2008; Hassan and Kassaify, 2014; Matabaro et al., 2017). Food mycotoxin detoxification processes are still ineffective in terms of food safety and cost. Monitoring programs are by far the most important strategy for reducing the risk of aflatoxins exposure in both animals and humans (Hassan and Kassaify, 2014).

The annual cow milk production throughout Burdur city is 378935 tone (TSI, 2018). It is an important centre for the country in terms of its raw milk production and most of milk is transferred to industry and processed and offered for consumption. Because of the public health concerns and the economic losses caused by the presence of aflatoxin in food, this study was conducted to detect the AFM_1 level in the milk samples gathered from dairy farms in Burdur province, assess the exposure of consumers, and estimate the risk.

MATERIAL and METHOD

Sample collection

In this study, 82 raw cow milk samples were randomly collected from farms in Burdur province of Türkiye, between September and December 2019. The samples were taken to the laboratory for analysis in covered sterile containers, under aseptic conditions and cold chain and kept at -18ºC further analysis.

Sample preparation

The presence of AFM_1 in the milk samples was detected by ELISA method, using Bio-Shield M_1 ES B2048/B2096 ELISA test kit according to the manufactures' instructions (Prognosis Biotech S.A., Larissa, Greece, 2019). The samples were centrifuged at 10°C, 3000xg for 10 minutes. After centrifuging, the upper cream layer was separated by a Pasteur pipet. The fat-free supernatant was used in the analysis. The dilution factor was calculated to be 1 for the milk samples prepared in this way.

Analysis procedure

The AFM_1 standards ranged in concentration from 0 to 250 ng kg^{-1}, and the milk samples were added at a rate of 100 µL per well in the microplate using an automated pipet. Then, the wells were covered with a transparent film and shaken for 30 seconds and were kept at room temperature for 45 minutes. Later, the liquid in the wells was discharged and the wells were irrigated 4 times with an irrigation buffer solution (Wash Buffer 1X). After each irrigation, the micro-plates were turned upside down, an absorbing paper was tapped on it in order to remove the liquid completely from the wells. After these steps, 100 µL AFM_1 Detection solution (AFM_1-HRP) was added to all of the wells. Then, the wells were covered with a transparent film and shaken for 30 seconds and were kept for 15 minutes at room temperature. The wells were emptied again and they were irrigated 4 times with irrigation solution (Wash Buffer 1X) and after each irrigation, the microplates were turned upside down and tapped. Subsequently, 100 µL of TMB Substrate was added to all of the wells. The wells were covered with transparent film and they were manually shaken for a few seconds incubated in the the dark at room temperature for 15 minutes. At the end of this process, 100 µL stop solution (15% H_3PO_4) was added to all of the wells. The plate was shaken by hand again and mixed slightly. The blue colour became yellow by adding a stopping solution to the wells. Then, it was measured in absorbance ELISA reader at 450 nm and in 60 minutes.

Assessment

The results obtained were assessed according to the computer program (Prognosis-Data-Reader) designed by Bio-Shield. The levels of the aflatoxin standards ranged from 0 to 250 ng kg^{-1}. According to the analysis preparation document, LOD value was 2 ng kg^{-1} and LOQ value was 5 ng kg^{-1} for milk. For milk, the recovery rate was 99.4% and the satisfactory range was 79-119% (Prognosis Biotech S.A., Larissa, Greece, 2019). The statistical analyses were conducted using Minitab for Windows Version Release 16.1. (Minitab Inc., 2011). Occurrence the AFM_1 in raw cow milk is reported as the mean±standard error, range (minimum–maximum), frequency distribution of samples and percentage of samples exceeding maximum limits of Regulations.

The assessment of the estimated exposure levels of consumers to AFM1

While assessing the exposure levels of population to AFM_1 in Türkiye, the calculations were made by considering the report published by World Health Organization (WHO, 2005). Estimated of daily intake (EDI) of population by milk consumption was calculated using the formula: EDI (ng kg^{-1} b.w. day^{-1}) = toxin (ng kg^{-1}) x milk consumption (kg $person^{-1}$ day^{-1})/body weight (kg).

The milk consumption quantity used to calculate the EDI was obtained according to the National Milk Council (NMC, 2018). Despite a lack of statistics on milk consumption by age group in Türkiye, a per capita milk consumption of roughly 270 kg $person^{-1}$ $year^{-1}$ is estimated (NMC, 2018). The daily consumption amount was estimated by dividing the total annual amount by 365. In this study, the mean body weight (b.w.) of 60 kg for adults population in Türkiye was used for calculating the EDI.

RESULTS and DISCUSSION

In this study, a total of 82 milk samples were analysed to determine AFM_1 concentration and the results obtained are shown in Table 1. It was determined that the AFM_1 level was over 5 ng kg^{-1} in 48 (58.5%) and the AFM_1 level was below 5 ng kg^{-1} in 34 (41.5%) among 82 milk samples gathered from several dairy farms in Burdur. The AFM_1 level was between 5.06 and 50.63 ng kg^{-1} in 48 (58.5%) of 82 raw cow milk samples analysed and the average contamination rate was 15.53±1.49 ng kg^{-1}. The AFM_1 contamination was 50.63 ng kg^{-1} in 1 (1.2%) of the milk samples.

Various studies have been conducted in different countries using different techniques in order to determined the presence and level of AFM_1 in milk. Different AFM_1 levels in raw milk samples found in the previous studies conducted in Türkiye and different countries were summarized in Table 2 and Table 3.

In this study, AFM_1 was detected in 58.5% of the raw milk samples. When compared to the previous studies conducted in Türkiye, this result was determined to be higher than the values determined by Keskin et al. (2009), and Aksoy and Sezer (2019), and lower than the values determined by Oruc et al., (2011), Ertas et al., (2011), Buldu et al., (2011), Sahindokuyucu Kocasari et al., (2012), Bakirdere et al., (2014), Temamogullari and Kanici (2014), Isleyici et al., (2015), Yildirim et al., (2018), Eker et al., (2019), Turkoglu and Keyvan (2019), and Guven et al., (2020).

Table 1. Occurrence and distribution of AFM_1 in raw cow milk samples collected from Burdur.
Çizelge 1. Burdur'dan toplanan çiğ inek sütü örneklerinde AFM_1'in varlığı ve dağılımı.

	Tested *Test edilen* n	Positive *Pozitif*	Distribution of samples *Örneklerin dağılımı* ng kg^{-1} (%)				Exceed legal limit** *Yasal sınırı aşan*** n (%)	AFM_1 concentration *AFM_1 konsantrasyonu* (ng kg^{-1})		
		n (%)	<5*	5-25	26-50	>50		Mean *Ortalama* ± SE	Min.	Max.
cow milk *inek sütü*	82	48 (58.5)	34 (41.5)	41 (50)	6 (7.3)	1 (1.2)	1 (1.2)	15.53 ± 1.49	5.06	50.63

n: number of samples, *Distribution of negative samples, **TFC and EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, SE: Standard Error

Table 2. AFM_1 levels in raw cow milk analyzed in Türkiye.
Çizelge 2. Türkiye'de analiz edilen çiğ inek sütlerindeki AFM_1 düzeyleri

Location *Yer*	n	Positive *Pozitif* n (%)	Range *Aralık* (Mean) (*Ortalama*)	Exceed legal limit *Yasal sınırı aşan* n (%)*	Reference *Kaynak*
İstanbul	60	20 (33.3)	5.40-300.20 (166.80) ng L^{-1}	5 (8.3)	Keskin et al. (2009)
Bursa	30	30 (100)	2.48-18.93 (7.23) ng kg^{-1}	0 (0.00)	Oruc et al. (2011)
Kayseri	50	43 (86)	1-30.0 (8.73) ng kg^{-1}	0 (0.00)	Ertas et al. (2011)
Kayseri	90	90 (100)	54.4-65.5 (59.93) ng L^{-1}	63 (70)	Buldu et al. (2011)
Burdur	45	41 (91.1)	15.3-80 (45.3) ng L^{-1}	16 (35.5)	Sahindokuyucu Kocasari et al. (2012)
Kocaeli, Sakarya, Düzce	77	61 (79.22)	0.005-0.410 (0.031) µg L^{-1}	4 (n.r.)	Bakirdere et al. (2014)
Şanlıurfa	38	n.r. (94.7)	0.82-125.70 (56.74) ng kg^{-1}	21 (55.3)	Temamogullari and Kanici (2014)
Van	100	85 (85)	<5->80 (n.r.) ng L^{-1}	12 (12)	Isleyici et al. (2015)
Kırıkkale	154	154 (100)	0.08-10.11 (1.73) ng L^{-1}	0 (0.00)	Yildirim et al. (2018)
Kars	50	28 (56)	0-21.57 (10.02) ng L^{-1}	0 (0.00)	Aksoy and Sezer, (2019)
Çanakkale	120	107 (89.2)	5.14-78.69 (16.70) ng kg^{-1}	4 (3.3)	Eker et al. (2019)
Burdur	35	35(100)	n.r. (25.45) ng L^{-1}	5 (14.28)	Turkoglu and Keyvan (2019)
Kars	80	80 (100)	0.00-17.86 (9.28) ng kg^{-1}	0 (0.00)	Guven et al. (2020)

n: No. of raw cow milk samples, *TFC and EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, n.r.: results not reported by author.

Table 3. AFM_1 contamination in raw cow milk analysed by ELISA in different countries.
Çizelge 3. Farklı ülkelerde ELISA ile analiz edilen çiğ inek sütündeki AFM_1 kontaminasyonu.

Country *Ülke*	n	Positive *Pozitif* n (%)	Range (*Aralık*) (mean) (*Ortalama*)	Exceed legal limit *Yasal sınırı aşan* n (%)*	Reference *Kaynak*
Serbian	40	38 (95)	0.005-0.90 (0.19) µg kg^{-1}	5 (12.5)	Kos et al. (2014)
Iranian	45	22 (48.88)	6.3-23.3 (11.61) ng L^{-1}	0 (0.00)	Zanjani et al. (2015)
Iranian	288	163 (56.59)	0.01-0.25 (0.95) ppb	113 (69.32)	Mahmoudia and Norian (2015)
Macedonia	3635	1538 (42.4)	<6.6-408 (14.3) ng kg^{-1}	105 (2.9)	Dimitrieska-Stojkovi et al. (2016)
Egypt	15	5 (33.3)	6.40-70 (35.68) ng L^{-1}	2 (13.3)	Tahoun et al. (2017)
Italy	416	51 (12.3)	n.r. (0.037 µg kg^{-1})	1 (n.r.)	De Roma et al (2017)
Pakistan	156	143 (91.7)	20-3090 (317.4) ng L^{-1}	125 (80.1)	Asghar et al. (2018)
China	133	100 (75.2)	5.3 -36.2 (15.9) ng L^{-1}	0 (0)	Xiong et al. (2020)
Lebanon	701	412 (58.8)	0.011-0.440 (0.035) µg L^{-1}	196 (28)	Daou et al. (2020)

n: No. of raw cow milk samples,*EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, n.r.: results not reported by author.

When comparing the AFM_1 level determined in the current study with the studies conducted in the other countries, the AFM_1 level determined in the current study was found to be lower than the values determined by Kos et al., (2014), Asghar et al., (2018), Xiong et al., (2020), and Daou et al. (2020) and higher than the values determined by Zanjani et al., (2015), Mahmoudia and Norian (2015), Dimitrieska-Stojkovi et al., (2016), Tahoun et al., (2017), and De Roma et al., (2017).

In this study, the average contamination level of AFM_1 in the milk samples was determined to be 15.53±1.49 ng kg^{-1}. The result obtained in this study was similar to the average contamination levels determined by Xiong et al., (2020). It was determined to be contaminated with high level of AFM_1 in comparison with the results obtained by Oruc et al., (2011), Ertas et al., (2011), Zanjani et al., (2015), Dimitrieska-Stojkovi et al., (2016), Yildirim et al., (2018), Aksoy and Sezer (2019), and Guven et al., (2020) and with low level of AFM_1 in comparison with the results obtained by Keskin et al., (2009), Buldu et al., (2011), Sahindokuyucu Kocasari et al., (2012), Bakirdere et al., (2014), Kos et al., (2014), Temamogullari and Kanici (2014), Mahmoudia and Norian (2015), Tahoun et al., (2017), De Roma et al., (2017), Asghar et al., (2018), Eker et al., (2019), Turkoglu and Keyvan (2019), and Daou et al., (2020). In comparison with the previous studies, it was determined that the AFM_1 levels in raw milk were variable. The AFM_1 residue levels in milk vary significantly based on the species of animal from which milk is obtained, milking type and time, lactation period, type, growing and keeping method of forage, geographical conditions (local weather, humidity and temperature), seasonal changes and the development levels of the countries (Galvano et al., 1996; Tajkarimi et al., 2008; Kamkar et al., 2014; Milićević et al., 2017; Akbar et al., 2019).

The presence of AFM_1 in milk and its products consumed in developing countries is a serious problem (Prandini et al., 2009). Due to high toxicity and health concerns, many countries have determined maximum residue limits for AFM_1 to prevent or reduce aflatoxin risks. According to Turkish Food Codex (TFC, 2011) and European Union's Regulation (EC, 2010), the maximum AFM_1 level in milk to be used in the production of raw milk, heat-treated milk and milk-based products must be 50 ng kg^{-1} or less. In the USA, according to FDA regulations, the AFM_1 level in milk must be 500 ng kg^{-1} or lower (FDA, 2005). In this study, it was determined that the AFM_1 amount of 1 (1.2%) of the milk samples exceeded the level of 50 ng kg^{-1} specified by Turkish Food Codex and European Union's Regulation, although 58.53% of the milk samples gathered from dairy farms were contaminated with AFM_1. In contradistinction to this study, it has been reported that the AFM_1 levels in the milk samples tested by Oruc et al., (2011), Ertas et al., (2011), Zanjani et al., (2015), Yildirim et al., (2018), Aksoy and Sezer (2019), Guven et al., (2020), and Xiong et al., (2020) have not exceeded the acceptable limits determined by European Union's Regulation. Some other researchers have declared that the AFM_1 levels (2.9-80.1%) determined in milk samples are higher than the level specified by the regulation (Keskin et al., 2009; Buldu et al., 2011; Sahindokuyucu Kocasari et al., 2012; Temamogullari and Kanici 2014; Bakirdere et al., 2014; Kos et al., 2014; Mahmoudia and Norian 2015; Isleyici et al., 2015; Dimitrieska-Stojkovi et al., 2016; Tahoun et al., 2017; Asghar et al., 2018; Eker et al., 2019; Turkoglu and Keyvan 2019; Daou et al., 2020). The fact that 48 milk samples (58.5%) determined to be contaminated with AFM_1 in this study were lower than the maximum residue limits did not mean that they were safe.

According to the current survey, the average level of AFM_1 is 15.53 ng kg^{-1}. Although the lack of data regarding the consumption of milk according to different age groups in Türkiye, drinking milk consumption is estimated to be approximately 270 kg person^{-1} year^{-1}, which equates to 0.74 kg of milk per day (NMC, 2018). In the light of these, based on the results obtained in the present study, the EDI of AFM_1 for Turkish adults was calculated 0.19 ng kg^{-1} b.w. day^{-1}, assuming an adult body weight of 60 kg.

There are no sufficient data available about the EDI values and exposure risks to AFM_1 by adult consumers in Türkiye. In the this study, the EDI of AFM_1 for adults was calculated 0.19 ng kg^{-1} b.w. day^{-1}. JECFA (2001) stated that the mean EDI of AFM_1 in milk was 0.11 ng kg^{-1} b.w. day^{-1} in European, 0.058 ng kg^{-1} b.w. day^{-1} in Latin America, 0.20 ng kg^{-1} b.w. day^{-1} in Far East, 0.10 ng kg^{-1} b.w. day^{-1} in Middle East, and 0.0020 ng kg^{-1} b.w. day^{-1} in Africa. This value in present study is 1.73, 3.28, 0.95, 1.90, and 9.5 times higher than the EDI determined for European, Latin America, Far East, Middle East, and Africa, respectively. The most of the previously EDI of AFM_1 through milk consumption in some countries were summarized in Table 4.

Tablo 4. Consumption of milk in different countries, average contamination, and exposure level to AFM_1.
Çizelge 4. Farklı ülkelerde süt tüketimi, ortalama kontaminasyon ve AFM_1'e maruz kalma düzeyi.

Country *Ülke*	Consumption of milk *Süt tüketimi*	Body weight *Vücut ağırlığı* (kg)	Mean AFM_1 concentration *Ortalama AFM_1 konsantrasyonu* (ng kg^{-1})	Mean EDI of AFM_1 *AFM1'in Ortalama EDI'si* (ng kg^{-1} b.w. day^{-1})	Reference *Kaynak*
Brazil	350 mL day^{-1}	60	31	0.188	Shundo et al. (2009)
Spain	adult male: 0.305 kgday^{-1} adult female: 0.305 kg day^{-1}	adult male: 80.83 adult female: 66.42	9.69	adult male: 0.036 adult female: 0.043	Cano-Sancho et al. (2010)
Portuguese	87 kg year^{-1}	69	23.4	0.08	Duarte et al. (2013)
Türkiye	71 g day^{-1}	60	46	0.054	Golge (2014)
Serbia	adult male: 0.21 L day^{-1} adult female: 0.18 L day^{-1}	adult male: 90 adult female: 69	210	adult male: 0.49 adult female: 0.56	Kos et al. (2014)
Brazil	350 mL day^{-1}	60	21	0.120	Santili et al. (2015)
Pakistan	adult male: 0.39 L day^{-1} adult female: 0.40 L day^{-1}	adult male: 79.3 adult female: 52.6	summer:94.9 winter: 129.6	adult male: 0.63 adult female: 1	Iqbal et al. (2017)

In comparison with previous studies, this result is higher than those detected by Shundo et al. (2009), Cano-Sancho et al., (2010), Duarte et al. (2013), Golge (2014), and Santili et al. (2015). However, this result is lower than reported by Kos et al. (2014), Skrbic et al. (2014), and Iqbal et al. (2017). Skrbic et al. (2014) reported that the mean AFM_1 exposure level for Serbian population through milk consumption, estimated at 1.420, 0.769 and 0.503 ng kg^{-1} b.w. day^{-1} in February, April and May, respectively. Kuiper-Goodman (1990) stated that the tolerable daily intake of AFM_1 was 0.2 ng kg^{-1} b.w. day^{-1}. However, the mean EDI of AFM_1 by adult consumer (0.19 ng kg^{-1} b.w. day^{-1}) was close to the calculated tolerable daily intake of 0.2 ng kg^{-1} b.w. day^{-1} of AFM_1. The international expert committees (JECFA, 2001) have concluded that the daily exposure causes the risk of liver cancer even in the concentration lower than 1 ng kg^{-1} although they have not determined a tolerable daily intake for aflatoxins. Considering this information, it is seen that the AFM_1 taken through milk causes a high risk in all age groups. Therefore, it is recommended to take the AFM_1 at the lowest levels "*As Low As Reasonably Achievable*" principle by experts on the subject (EFSA, 2004). Milk is not only source of AFM_1. Additionally, it can be found in commonly consumed dairy products, such as yogurt, cheese, and milk-based desserts. Therefore, additional studies are required about commonly consumed dairy products to accurately predict consumers' exposure to AFM_1. New strategies are needed to reduce exposure to aflatoxins, especially AFM_1.

CONCLUSION

Consequently, it was satisfactory that the AFM_1 level determined in the milk samples analyzed in this study was lower than the maximum tolerance level determined in TFC and EU Regulation. However, the presence of AFM_1 in 58.5% of the milk samples poses a risk to public health. For this reason, continuous monitoring of AFM_1 at every stage should be performed for animal health, public health and the economy of the country. In order to prevent the formation of aflatoxin, the forage and foodstuff must be produced and kept under appropriate conditions. Also, it is essential to train the producers and consumers about the dangers of aflatoxins and the measures to be taken to minimize the contamination.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

No potential conflict of interest was reported by the authors.

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