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RESEARCH ARTICLE

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Detection of Ochratoxin A In Bulk Tank Milk

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ABSTRACT

Ochratoxin A (OTA) produced by several *Aspergillus* and *Penicillium* species is a mycotoxin that contaminates different foods and feedstuffs, including cereals, coffee beans, nuts, cocoa, pulses, beer, wine, spices, dried vine fruits, meat, milk. In humans and animals, OTA has been observed to be particularly nephrotoxic, hepatotoxic, immunotoxic, neurotoxic, embryotoxic, carcinogenic and teratogenic. Ochratoxin A is a stable molecule and can remain unchanged even after the processes applied. In this study, it was aimed to determine the presence of ochratoxin A in milk samples (n:40) collected from bulk tank milks in Burdur province of Turkey. The presence of OTA in the samples was analyzed by using ELISA. The analyzes were performed according to the manufacturer's instructions. As a result, Ochratoxin A was found in 40 cow's milk samples (range 2-270 ng/l) collected from bulk milk tanks. The results of this study show that cow's milk should be considered as a potential OTA source in the human diet. It is proposed to examine the presence of OTA more intensively in dairy products and to determine their maximum limit values by conducting necessary studies.

Keywords: Burdur, Ochratoxin A, Milk, Mycotoxin

Süt Toplama Tanklarında Okratoksin A Varlığının Belirlenmesi

ÖZ

Okratoksin A (OTA), *Aspergillus* ve *Penicillium* türü mantarlar tarafından sentezlenen ve tahıl, kahve çekirdeği, fındık, kakao, bakliyat, bira, şarap, baharat ve kuru üzümde bulunabilen bir mikotoksindir. İnsanlarda ve hayvanlarda, OTA özellikle nefrotoksik, hepatotoksik, nörotoksik, embriyotoksik, immunotoksik, teratojenik ve karsinojenik etkiler gösterir. Okratoksin A kısmen kararlı bir moleküldür ve gıdalara uygulanan işlemlerden sonra bile değişmeden kalabilir. Bu çalışmada; Burdur bölgesinde bulunan süt toplama tanklarında (n:40) OTA varlığının belirlenmesi amaçlandı. Örneklerde OTA varlığı ELISA kullanılarak analiz edildi. Analizler üreticinin talimatlarına göre yapıldı. Süt toplama tanklarından alınan 40 inek süt örneğinde (2-270 ng/l aralığında) OTA bulundu. Bu çalışmanın sonuçları, inek sütünün insan beslenmesinde potansiyel bir OTA kaynağı olarak görülmesi gerektiğini göstermektedir. OTA varlığını süt ürünlerinde daha yoğun bir şekilde incelenmesi ve gerekli mevzuat çalışmaları yapılarak maksimum limit değerlerinin belirlenmesi önerilmektedir.

Anahtar kelimeler: Burdur, Okratoksin A, Mikotoksin, Süt

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INTRODUCTION

Mycotoxins produced by *Fusarium, Aspergillus* and *Penicillium* cause toxic effects on humans and animals by consumption of mycotoxin-contaminated foods such as cereals, corn, fruits, milk, egg and feeds such as grain (Capriotti et al., 2012; Binder, 2007; García-Moraleja et al., 2015). Milk is one of the most important sources of animal protein in human nutrition. Especially for the children who are in the age of growth should consume milk for adequate and balanced nutrition. Therefore, it is important that milk shouldn't contain harmful toxic components for human health especially for children who are more sensitive for toxins rather than older people (Flores-Flores et al., 2015). Although many studies have demonstrated that aflatoxin M_1 is the most common mycotoxin in milk, some researchers have also detected other mycotoxins such as ochratoxin A (OTA), fumonisin, aflatoxin G_1 (Herzallah, 2009; Gazzotti et al., 2009; Huang et al., 2014).

Ochratoxin A is a mycotoxin that produced by *Aspergillus* and *Penicillium* species. It can be found in spices, raisins, cereal, coffee beans, nuts, cocoa, pulses, beer and wine (Varga et al., 2006). Ochratoxin A has immunotoxic, nephrotoxic, embryotoxic, teratogenic, neurotoxic, hepatotoxic, genotoxic, and carcinogenic effects (Weidenbach et al., 2004; Malir et al., 2013). As OTA is a stable compound, high temperatures (above 250 °C) are required to decrease the toxin levels. It is not destroyed by common food preparation process (Boudra et al., 1995). It was classified by Agency for Research on Cancer as group 2B (possibly carcinogenic to humans) (IARC, 1993). Therefore, OTA's Provisional Tolerable Weekly Intake (PTWI) is 120 ng/kg of body weight (bw) according to the European Commission. Contamination of OTA is receiving increasing attention worldwide, owing to possible harmful effects on human and animal health (Keyvan and Yurdakul, 2015). Animal origin food products contaminated by OTA can create a risk to human health. For this reason, animal origin food products like meat and milk should be analyzed in order to detect OTA contamination (Duarte et al., 2012). The aim of this work was to detect the potential presence of ochratoxin A in bulk tank milks collected from Burdur province of Turkey.

MATERIAL and METHODS

Milk samples

A total of 40 bulk tank milk samples were obtained from Bulk milk tanks in Burdur province, located in the southern side of Turkey from July to October 2017. The milk samples were stored at -20 °C until they were used.

Determination of OTA in the milk

After the samples reached room temperature, 750 µl of methanol was added to 250 µl of the milk sample. The mixture was stirred at room temperature for 5 min. Subsequently, centrifugation was performed and the supernatant was used for analysis. For analysis of OTA, Ochratoxin A Serum/Milk ELISA test kit (Helica Biosystem Inc; 9410CH01M-96) was used and analyzes were performed according to the manufacturer's instructions.

Statistic

The standard curve was prepared according to the manufacturer's instructions.

RESULTS

In the current study, the contamination of OTA in bulk milk tank milk collected from bulk tank milk in Burdur province of Turkey was detected by ELISA. The standard curve was linear with a determination coefficient (R^2) of 0.969 for OTA (Figure 1). Ochratoxin A was found in 40 cow's milk samples (range 2-270 ng/l) (Figure 2).

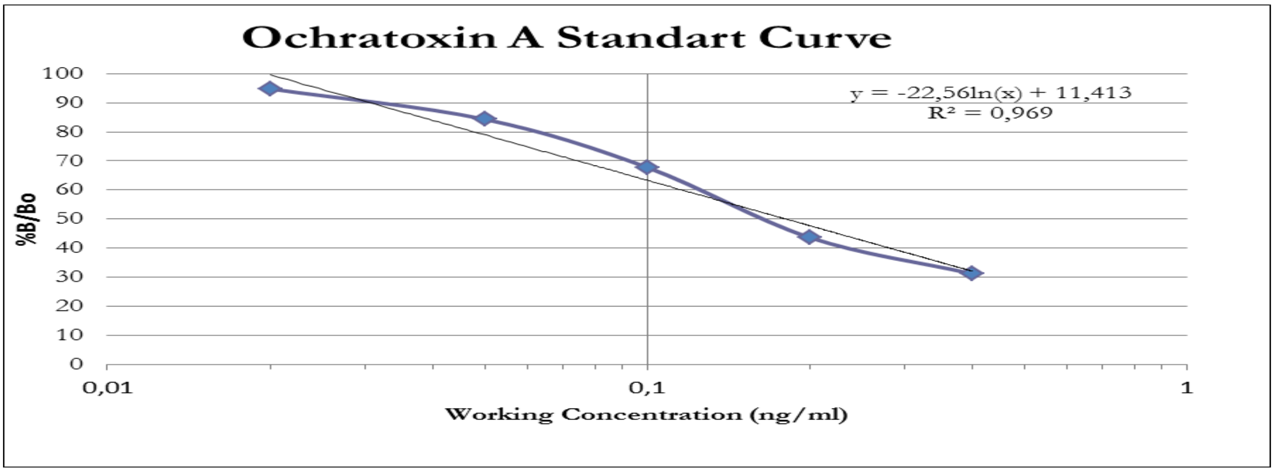

Figure 1. Ochratoxin A standard curve and equation.

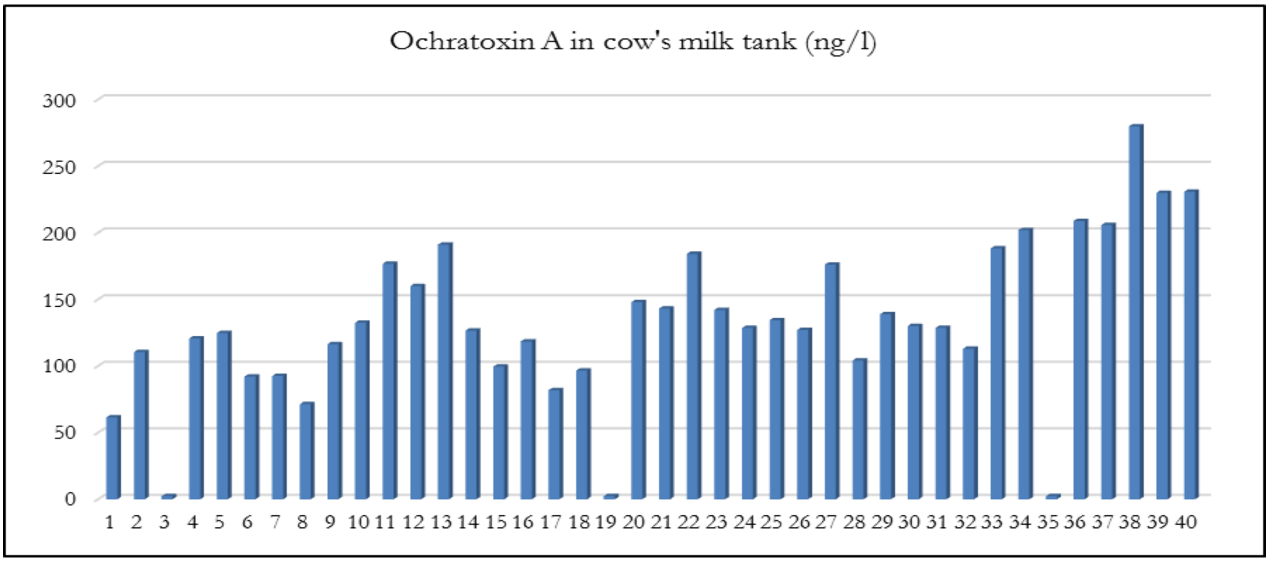

Figure 2. Levels of Ochratoxin A in cow's milk sample collected from bulk milk tanks in Burdur Province.

DISCUSSION

Ochratoxins have been found in wide range of foods and feedstuffs. Ochratoxin A (OTA) is the most important toxin in groups of Ochratoxin which can be also found animal-derived products, such as meat and milk (Alshannaq and Yu, 2017). In a study, OTA was detected in 6 out of 40 conventional cow's milk samples (range 11-58 ng/l), and in 5 out of 47 organic milk samples (range 15-28 ng/l) in Norvey (Skaug, 1999). Ochratoxin A was found in 5 out of 36 cow's milk samples (range 10-40 ng/mL) collected from Sweden (Breitholtz-Emanuelsson et al. 1993). However, no OTA was detected in the samples in samples (n=121) of cow's milk obtained from a northern region of Germany (Valenta and Goll, 1996). In all three studies, the detection limit was set at 10 ng/l. In this study, OTA was detected in 37 out of 40 cow's milk samples (range 10-270 ng/l) collected from milk collection tanks in Burdur province of Turkey. Differences observed in the results of the studies can be attributed to the fact that the weather conditions during growth, harvesting and storage of crop have a great influence on OTA levels (Jørgensen et al., 1996). Presumably, the amount of OTA in the feed, which is dependent on these factors, changes the OTA level in the milk. Differences in climatic and husbandry procedures may explain the changes in OTA contamination between countries and different farms.

According to Regulations (EC) No. 1881/2006 and 105/2010, maximum levels (µg/kg) for OTA in foodstuff have been established to minimize exposure of the public in European Union. Some of European Union (EU) members constrict the limits or set limits in commodities not specified by the European Union harmonized guidelines in some cases. Slovakia set a limit of 5 µg/kg for milk although there are no regulations in other countries with EU for OTA in milk (Duerta et al. 2010). The OTA levels in this study were well below the limit values of Slovakia which set the OTA limit. However, when it is evaluated in the aspect of tolerable daily intake (TDI) or tolerable weekly intake (TWI), OTA levels found in this study was higher than the maximum limit of OTA calculated. A TWI of OTA has been reported by the European Food Safety Authority as 120 ng/kg bw in 2006 (EFSA, 2006). The Nordic Working Group (1991) has suggested a TDI of OTA in humans of 5 ng/kg bw. Assuming that a child (4-years-old) is 15 kg and consumes about 400 ml milk every day, no more than 200 ng/l OTA must be present in the milk according to the Nordic Working Group. However, in this study, 6 (15%) out of 40 positive samples showed more than the maximum limit of 200 ng/l OTA. The OTA levels in cow's milk (15%) found in this study are adequate to lead to a higher intake of OTA than the proposed TDI of 5 ng/kg bw in children consuming large amounts of milk.

Risk assessments made do not differentiate between risk group, especially children, and adult groups (Skaugh 1999). Children represent a particularly sensitive population group in which a specific TDI should be assessed, particularly considering the inappropriate dose/body weight ratio. The presence of contaminants in cow's milk is likely to have greater impacts on infants and children than adults who can be fed on a more diverse diet. The results of this study and other studies demonstrate that cow's milk should be considered as a potential OTA source in the human diet. It is proposed to examine the presence of OTA more intensively in dairy products and to determine their maximum limit values by conducting necessary studies.

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