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Full Length Research Paper

Determination of zearalenone contamination in wheat and rice in Chaharmahal va Bakhtyari, Iran

Mojtaba Rashedi[1,2]*, Mohammad Ali Ashjaazadeh[2], Hamid Reza Sohrabi[2], Hamed Azizi[2] and Ebrahim Rahimi[1,3]

[1]Young Researcher Club Office, Faculty of Veterinary Medicine, Islamic Azad University of Shahrekord Branch, Shahrekord, Iran.
[2]Scientific Association of Veterinary Medicine Office, Faculty of Veterinary Medicine, Islamic Azad University of ShahreKord Branch, Shahrekord, Iran.
[3]Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

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Zearalenone (Mycotoxin F2) is a mycotoxin produced by genus *Fusarium* in cereals. This mycotoxin is a non-steroidal metabolite with esterogenic-like effects that causes reproductive problems in animals specially swine (infertility, abortion) and hyperestrogenism in women. The current study explores zearalenone contamination in wheat and rice. A total of 35 samples were collected from ShahreKord (Chaharmahal va Bakhtiari Province) in spring and summer 2010, which contained 15 wheat samples and 20 rice samples. The samples were analyzed by using direct competitive enzyme-linked immunosorbent assay (dcELISA). In 35 analyzed samples, zearalenone was found in 2 samples (5.7%) with a mean level of 89 µg kg^{-1}. Only in rice samples zearalenone were detected (10%), while there was not any contamination in wheat samples. The results of this study show zearalenone from exposed wheat and rice would not be of health concern for Public Health in Iran.

Key words: Zearalenone (ZEN), wheat, rice, direct competitive enzyme-linked immunosorbent assay (dcELISA), Iran.

INTRODUCTION

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus*, *Penicillium* and *Fusarium* (Zinedine et al., 2006). Currently, more than 400 mycotoxins are identified in the world. Considering their heat stability, these substances constitute a potential risk for human and animal health. The chemical and biological properties of mycotoxins and their toxic effects are extremely variable. These negative effects include carcinogecity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity and immunotoxicity (Zinedine and Manes, 2009) and females being more sensitive than males (EFSA, 2011). Mycotoxins are not only dangerous for the Public Health, but they also deteriorate the marketable quality of the contaminated products, causing tremendous economic losses (Zinedine and Manes, 2009).

The structure of zearalenone (ZEN) was first determined by Urry et al. (1966). ZEN is the generic name for [6- (10- hydroxyl-6-oxo-trans-1- undecenyl)-B-resorcylic acid lactone], which is a potent estrogenic metabolite produced by several species of *Fusarium* fungi and recognized to be a common contaminant in cereal grains and animal feed stuffs (Prelusky et al., 1989). It is found worldwide in a number of cereal crops such as maize, barley, oats, wheat, rice and sorghum (FAO, 2002) and also in bread (Aziz et al., 1997). It is insoluble in water and heat stable (Songsermsakul et al., 2006). Various health disorders (including loss of body weight, infertility, vaginal prolapse and enlargement of the uterus, vulva and mammary glands) associated with the intake of this

*Corresponding author. E-mail: mojtaba.rashedi@yahoo.com. Tel: (+98) 913-3124600. Fax: (+98) 311-3601493.

Abbreviations: dcELISA, Direct competitive enzyme-linked immunosorbent assay; **ZEN,** zearalenone.

mycotoxin have been well documented in domestic animals (Prelusky et al., 1989). Furthermore,studies under experimental conditions reported that ZEN could be potentially carcinogenic compound (Prelusky et al., 1989). ZEN causes alterations in the reproductive tract of laboratory animals (mice, rat, guinea-pigs, hamsters, rabbits) and domestic animals (JECFA, 2000). Various estrogenic effects like decreased fertility, increased embryolethal resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and estradiol have been observed but no teratogenic effects were found in mice, rats, guinea pigs and rabbits (JECFA, 2000). It also reported that ZEN causes hyperoestrogenism in farm animals, especially in pig (MacDonald et al., 2005). The presence of ZEN in foodstuffs may cause hyperestrogenism in women that may result in numerous manifested systemic disorders such as decreased libido, ovarian and uterine dysfunctions, anovulation, infertility and neoplasmic lesions in the entire reproductive system (Tiemann et al., 2003). Further data showed that ZEN was associated to endometrial adenocarcinomas (Tomaszewski et al., 1998).

The purpose of this survey was to detect the levels of ZEN in wheat and rice sampled at different locations in Chaharmahal va Bakhtyari Province in Iran, as well as to compare these levels with maximum ZEN limits adopted by European, USA, and Iran health organizations.

MATERIALS AND METHODS

Sample preparation

A total of 35 samples (15 of wheat and 20 of rice), were purchased from different locations in Chaharmahal va Bakhtyari Province of Iran during the spring and the summer of 2010. All samples were ground with a household blender and kept at -18°C in zipper bags until analysis. Samples (10 g) were extracted twice with 25 ml methanol-water (8:2) for 30 min with an orbital shaker and the extract was centrifuged then at 500 g for 10 min. Supernatant (40 ml) was transferred into a 250 ml pear-shaped separately funnel to which 40 ml hexane was added. After shaking the contents for 2 min, the lower aqueous methanol phase was drained into a screw cap tube with a Teflon liner.

Sample extracts subjected to ELISA were diluted 10 to 50 fold with a 0.02 m Tris-buffered saline solution containing 0.05% Tween 20 (TBST, pH 7.4). The plate was washed with TBST three times and 50 µl ZEN standard or the diluted sample extract was added to each well followed by 50 µl ZEN-HRP conjugate in TBST. After 1 h incubation at ambient temperature, the plate was washed with TBST and 100 µl TMB substrate was added. The TMB solution was made by mixing 1 mg TMB dissolved in 1 ml methanol with 9 ml 0.045 M sodium citrate buffer (pH 5.0) containing 0.002% hydrogen peroxide. After a 30 min reaction time, 100 µl stop reagent (2 N sulphuric acid) was added to each well. Absorbance at 450 nm was measured with a microplate reader.

RESULTS

The results of our study about the ooccurrence of ZEN contamination in rice and wheat samples are presented in Table 1. ZEN was detected in only 2 samples of rice (5.7%) with a mean level of 86 µg kg^{-1} by direct Enzyme-Linked Immunosorbent Assay (dcELISA) method. These two ZEN-positive samples contained lower than the EU (100 µg kg^{-1}), US-FDA and ISIRI (200 µg kg^{-1}) accepted action level ZEN.

DISCUSSION

Previous studies have shown that contamination of cereals with ZEN is a serious problem for consumer health. In Spain, Ibanez-Vea et al. (2011) reported that 48% of breakfast cereal samples were contaminated with ZEN, with a mean level of 25.4 µg kg^{-1}. ZEN showed the highest contamination rates in the samples containing wheat and wheat and rice (Ibanez-Vea et al., 2011) and in Saudi Arabia ZEN was detected in 40% of the total samples. Only 25% of the local samples were positive to ZEN with a maximum level of 4,000 µg.kg^{-1}. The percentage of positive samples were higher in the imported wheat samples (50%), while ZEN maximum level reached 10,000 µg.kg^{-1} and the overall mean was 1663 µg.kg^{-1} compared to the local samples (Al-Hazmi, 2010). Moreover, in other investigation for Tunisian foods, revealed that around 15% of samples were contained with a mean level of 11.81 µg kg^{-1} ZEN while (Ghali et al., 2008) and from 51, 1.9% of wheat and derived products samples were contaminated with a mean level of 2.82 µg kg^{-1} (Ghali et al., 2008) and in Mexico City ZEN contamination was found in 71.4% of wheat samples (Gonzalez-Osnaya and Farres, 2011). In China, cereals samples contaminated with ZEN reported to be in a range of 5 to 1400 µg kg^{-1}, due to different weather conditions (Li et al., 2002), while in Qatar wheat samples contamination were low its range was 0.2 to 2 µg kg^{-1}. In addition, in India it has been reported an occurrence of ZEN contamination up to 0.6 µg kg^{-1} (JECFA, 2000), but the occurrence of ZEN in 15 wheat samples were 0%, due to season, measuring or storing methods, as well as moisture and temperature levels in silos or stores in factories producing flour.

The results of our study are in agreement with the findings of previous studies. For example, Ibanez-Vea et al. (2011) reported that 50% of rice samples were contaminated with mean level of 5.22 µg kg^{-1} of ZEN, and Park et al. (2005) detected ZEN in Korea in rice samples with a level of 21.7 to 47 µg kg^{-1}. However, other studies reported very low contamination in rice samples in a range of 0.18 to 1.4 ng g^{-1} in [Qatar Abdulkadar et al. (2004) and Ghali et al. (2008) had not been detected ZEN)] in rice samples. Mycotoxin contamination in rice is usually lower as in wheat or corn (Tanaka et al., 2007) but in the present study two rice samples from 20 totally samples (10%) were contaminated with a mean level of 89 µg kg^{-1}, indicating that contamination of rice with ZEN is not a serious problem in Chaharmahal and Bakhtiari

Table 1. Occurrence of zearalenone contamination in rice and wheat in Iran.

Sample	Number of sample	Number of positive sample	Maen (µg kg-1)*
Rice	20	2 (10%)	89
Wheat	15	0 (0%)	0
Total	35	2 (5.7%)	89

*analyzed by dcELIZA

Province. The previous finding shows that Iranian rice has been collected in good manner and has been stored in good situation. However, the two rice contaminated samples had been collected during the spring, while all negative samples had been collected during the summer. Previous studies have been supported that, warm weather conditions can reduce ZEN contamination (Amparo et al., 2004).

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