Journal of Food Quality and Hazards Control 1 (2014) 25-28

Occurrence of Zearalenone in raw animal origin food produced in North-West of Iran

R. Mahmoudi * (PhD)

Department of Food Hygiene and Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Article type
Original article

Keywords
Food
Zearalenone
Iran

Received: 2014-01-20
Revised: 2014-03-13
Accepted: 2014-03-25

ABSTRACT

Introduction: Zearalenone is a mycotoxin compound produced mainly by the *Fusarium* species of fungi which is present in several types of foods. The purpose of this study was to determine the zearalenone in raw animal origin food produced in North-West of Iran.

Materials and methods: From June to December 2012, a total of 210 samples (containing 70 raw milk, 70 meat and 70 liver) were obtained from female buffaloes in the North-West regions of Iran. Samples were analyzed by ELISA method.

Results: The zearalenone was found in 92 of the 210 samples (43.80%). Significant differences in the mean values of zearalenone was observed between milk, meat and liver samples (p<0.05). The highest mean level of zearalenone was observed in liver samples (2.37±1.18 ng/g), followed by milk (1.34±1.42 ng/ml) and meat (0.79±1.27 ng/g) samples. The overall contamination rate during autumn was significantly more than summer (p<0.05).

Conclusion: The results of this study indicate that the occurrence of zearalenone contamination in the buffalo milk, meat and liver samples were low in this region of Iran, most probably because of the uncontaminated feed given to water buffalo. However, it seems that the most practical way to minimizing mycotoxin production and contamination of the food supply, is the development of methods to control their formation, or the development of newer methods to detoxify or decontaminate the affected food.

Introduction

Contamination of feeds and foods by mycotoxins is considered as one of the major factors related to safety for consumers. Mycotoxins represent different groups of secondary fungi metabolites which are most frequent contaminants of different grains and other feed components, such as alfalfa hay, sunflower, soy bean meal, etc (Creppy, 2002; Murphy et al., 2006). Maize and cereal crops, with relatively high share in livestock feeds, are potentially high risk factors towards health and production of livestock (cattle, buffalo, sheep, poultry, etc.), as these plant species are very susceptible to toxigenic mould species. For humans, food prepared from contaminated plants and animal origin products, such as milk, cheese and meat products have the highest risk of presence of mycotoxins (Krnjaja et al., 2009).

Zearalenone (ZEA) is a mycotoxin compound produced mainly by *Fusarium* species. Those fungi are present in worldwide cultivated cereals such as wheat, corn, oats, barley and rice (Marin et al., 2013; Zinedine et al., 2007). Great amounts of ZEA are produced by the *Fusarium* species during cereal storage under high humidity and temperature. This mycotoxin is a stable compound resistant to storage, milling, food processing and cooking (Mankeviciene et al., 2007).

The ZEA has a pronounced estrogenic action and numbers of animals are susceptible to it, but swine are particularly the most sensitive. It has been stated that ZEA may stimulate growth of cells with estrogenic receptors in human mammary glands. It is so supposed that ZEA may lead to breast cancer in human beings (Kennedy et al., 1998; Marin et al., 2013).

The US Food and Drug Administration (FDA) set tolerable daily intake of 0.2 µg/kg body weight for ZEA. Acceptable limits for ZEA in maize and other cereals, ranging from 50-1000 µg/kg, have been set in several countries in Europe, Asia, Africa and Latin America (FAO, 2004).

*Corresponding author
Email: mahmodi@tabrizu.ac.ir

Some studies have been done to detect ZEA in different kinds of food produced throughout the world (EFSA, 2011; El-Hoshy, 1999; Hewitt et al., 2012; Iqbal et al., 2014). But, there is no published data regarding analysis of ZEA in produced food of Iran.

Climatic conditions and growing of grains on large areas in North-West region of Iran are suitable for development of mould, and as a result, it seems that there is risk of animal feed contamination by their toxic products. Thus, this study was carried out to evaluate the occurrence of ZEA in raw animal origin food (milk, meat and liver) produced in North-West of Iran.

Materials and methods

Sampling

In 2012, a total of 210 samples of raw milk, meat and liver were obtained from 70 female buffaloes in three main cities located in the North-West regions of Iran including Tabriz (20 samples), Urmia (32 samples) and Ardabil (18 samples). All of the samples were transported to the laboratory at 2–4 °C in the icebox. The samples were categorized in two seasonal groups including summer (35 samples) and autumn (35 samples).

Method for analysis of ZEA

The quantitative analysis of ZEA in the milk samples was performed (Bennett et al., 1994) by competitive enzyme immunoassay using Zearalenone ELISA kit (Quantative EuroClone Zearalenone KIT, Cod. EEM007096. LOT. Z21423).

Preparation of milk samples

Preparation of milk samples was conducted according to the kit instructions. Milk samples were chilled to 10 °C and then centrifuged at 2000g for 5 min. The upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted supernatant) 200 µl was directly used per well in the test.

Preparation of meat and liver samples

In order to measure the amount of ZEA in meat and liver samples, the ELISA technique was performed according to manufacturer's instruction. In brief, 2 g of each sample was weighed and homogenized with mixer. Then, the homogenized samples were mixed with 10 ml of mixed solution of distilled water and methanol. The suspension was vortexed for 30 min and centrifuged at 3000 g for 10 min at room temperature. Then, the centrifuged supernatant solution was diluted at a ratio of 1/2. A 100 µl of the aqueous (upper) layer was used per well in the assay.

ELISA test procedure

ELISA test procedure was conducted according to the instructions of kit. At first, 200µl of standard solutions were provided in 0, 0.5, 2, 20 and 200 ng/l concentrations and the prepared samples were added into separate microplate wells and incubated for 30 min at room temperature (20–25 °C) in the dark. The liquid was then poured out and the wells were washed with washing buffer (250 µl) three time. In the next stage, 200 µl of the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark. Again, the wells were washed three time with washing buffer. Afterwards, 200 µl of substrate/chromogen was added, mixed gently and incubated in the dark at room temperature for 15 min. Finally, 50 µl of the stop reagent was added into the wells and the absorbance was measured at k = 450 nm in ELISA plate reader (Biotek Elx 808) against air blank within 15 min.

Statistical Analysis

The statistical analysis was based on normal confidence intervals and analysis of variance (one-way ANOVA) using SPSS software. The levels were considered significantly different at $p<0.05$.

Results

Concentration of ZEA in raw milk, meat and liver samples are shown in Table 1.

The toxin was detected in 92 of the 210 samples (43.80%). Significant differences in the mean values of ZEA was observed between milk, meat and liver samples ($p<0.05$). The highest mean level of ZEA was found in liver samples (2.37±1.18 ng/g), followed by milk (1.34±1.42 ng/ml) and meat (0.79±1.27 ng/g) samples. The toxin was detected in 92 of the 210 samples (43.80%). Significant differences in the mean values of ZEA was observed between milk, meat and liver samples ($p<0.05$).

Table 1: Level of ZEA in raw milk, meat and liver produced in North-West of Iran

Sample type	Sample size	Contaminated samples (%)	Concentration (mean ±SD) (ng/ml) (ng/g)	Range (ng/ml) (ng/g)
Milk	70	15 (21.42)	1.34±1.42[a*]	0.1-3.55
Meat	70	29 (41.42)	0.79±1.27[b]	0.1-2.5
Liver	70	48 (68.57)	2.37±1.18[c]	0.1-4.34
Total	210	92 (43.80)	1.49±1.34	0.1-4.34

*The mean values followed by the different letters in the column are significantly different ($p<0.05$)

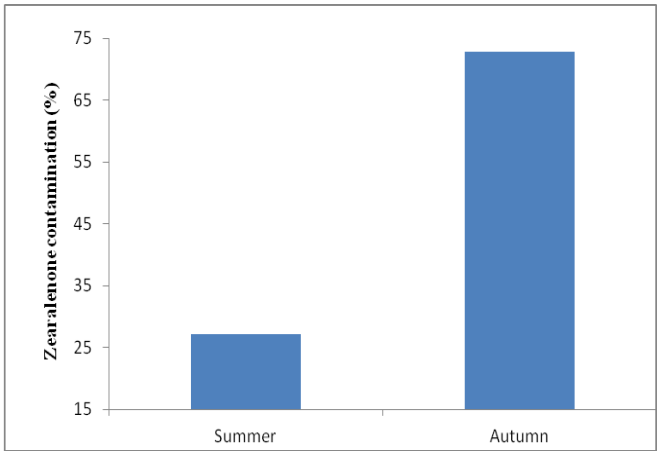

Fig.1: Zearalenone contamination in the raw animal origin food produced in North-West of Iran in different seasons

The highest mean level of ZEA was found in liver samples (2.37±1.18 ng/g), followed by milk (1.34±1.42 ng/ml) and meat (0.79±1.27 ng/g) samples. The overall contamination rate during autumn was significantly (p<0.05) more than summer (Fig. 1).

Discussion

Although most of the reported ZEA-contaminated foods are grain and cereal products, but the toxin may be found in animal origin food such as meat, milk, cheese, etc. (Kleinova et al., 2002). In the present study, considerable numbers 40.80% of buffalo liver, meat and milk samples were contaminated with ZEA.

According to the report of European Food Safety Authority (EFSA, 2011), ZEA was detected in 17%, 44% and 33% of grains, maize products and biscuit samples, respectively. Hewitt et al. (2012) observed ZEA in 22 out of 35 fresh corn and corn products in markets of San Diego County, USA.

According to a 2014 survey carried out in Punjab, Pakistan, 52% of 115 chicken meat and 32% of 80 egg samples were contaminated with ZEA and maximum level of 5.10 µg/kg was detected in the liver part of chicken meat (Iqbal et al., 2014). Similar research in Alexandria Governorate showed that 20% of each raw milk and meat samples were contaminated to this toxin. Also, the average concentration of ZEA in raw milk and meat samples were 6.9±1.46 µg/kg and 8.7±1.6 µg/kg, respectively (El-Hoshy, 1999).

The weather conditions (high air humidity and low temperatures) at harvesting contribute to an increase in the amount of ZEA produced by moulds (Krnjaja et al., 2009; Mankeviciene et al., 2007). Based on the finding of this study and considering especial climate of our study area, more ZEA concentration in the samples of autumn compared to summer ones seems to be logical.

Conclusion

The results of this study indicate that the occurrence of ZEA contamination in the buffalo milk, meat and liver samples were low in this region of Iran, most probably because of the uncontaminated feed given to water buffalo.

However, it seems that the most practical way to minimizing mycotoxin production and contamination of the food supply, is the development of methods to control their formation, or the development of newer methods to detoxify or decontaminate the affected food.

Conflicts of interest

None declared.

Acknowledgement

This work was financially supported by University of Tabriz, Tabriz, Iran. The author thanks Mr. Reza Norian for his valuable assistance.

References

Bennett G.A., Nelsen T.C., Miller B.M. (1994). Enzyme-linked immunosorbent assay for detection of zearalenone in corn, wheat, and pig feed: collaborative study. *Journal of AOAC International*. 77: 1500-1509.

Creppy E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*. 127: 19-28.

El-Hoshy S.M. (1999). Occurrence of zearalenone in milk, meat and their products with emphasis on influence of heat treatments on its level. *Archiv für Lebensmittelhygiene*. 50: 140-143.

European Food Safety Authority (EFSA), (2011). Scientific opinion on the risks for public health related to the presence of zearalenone in food. *EFSA Journal*. 9: 2197.

Food and Agriculture Organization (FAO), (2004). Worldwide regulations for mycotoxins in food and feed. Rome. p: 81.

Hewitt T.C., Flack C.L., Kolodziejczyk J.K., Chacon A.M., D'Ovidio K.L. (2012). Occurrence of zearalenone in fresh corn and corn products collected from local Hispanic markets in San Diego County, CA. *Food Control*. 26 : 300-304.

Iqbal S.Z., Nisar S., Asi M.R., Jinap S. (2014). Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs. *Food Control*. 43: 98-103.

Kennedy D.G., Hewitt S.A., McEvoy J.D., Currie J.W., Cannavan A., Blanchflower W.J. (1998). Zeranol is formed from Fusarium spp. toxins in cattle in vivo. *Food Additives and Contaminants*. 15: 393-400.

Kleinova M., Zollner P., Kahlbacher H., Hochsteiner W., Lindner W. (2002). Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. *Journal of Agricultural and Food Chemistry*. 50: 4769-4776.

Krnjaja V., Levic J., Stankovic S. (2009). Ubiquityof toxigenic fungi and mycotoxins in animal feeds in republic of Serbia. *Biotechnology in Animal Husbandry*. 25: 477-491.

Mankeviciene A., Butkute B., Dabkevicius Z., Suproniene S. (2007). Fusarium mycotoxins in Lithuanian cereals from the 2004–2005 harvests. *Annals of Agricultural and Environmental Medicine*. 14: 103-107.

Marin S., Ramos A.J., Cano-Sancho G., Sanchis V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*. 60: 218-237.

Murphy P.A., Hendrich S., Landgren C., Bryant C.M. (2006). Food mycotoxins: an update. *Journal of Food Science*. 71: 51-65.

Zinedine A., Soriano J.M., Molto J.C., Manes J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food and Chemical Toxicology*. 45: 1-18.