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ORIGINAL

Evaluation of nitrofuran content in eggs and milk supplied in Tehran, Iran

Evaluación del contenido de nitrofuranos en los huevos y la leche suministrados en Teherán, Irán

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Abstract

Background: The frequent use of antibiotics causes antibiotic residues in various food of animal origin, including milk and egg. Nitrofurans are used as feed additives to prevent and treatment of intestinal infections. However, the use of nitrofurans is prohibited in livestock due to carcinogenicity. This survey was designed to measure the concentration of nitrofuran residues in milk and eggs.
Methods: The samples were selected from the Tehran market, Iran. The detection was done with the enzyme-linked immunosorbent assay (ELISA) method. The sample preparation was performed according to the kit protocol.
Results: The residue was detected in two samples of milk at 216.6 and 208.1ng/ kg. However, the residue of the drug was not detected in any of the egg samples. The estimated dietary intake based on the mean concentration of milk samples was calculated at 0.000000031 mg/kg BW/day.
Conclusion: The calculated EDI is not significant, but it is necessary to evaluate the residual nitrofuran based on the milk data regularly.

Keywords: Dietary exposure, nitrofuran metabolite, residues, milk, egg.

Resumen

Introducción: El uso frecuente de antibióticos provoca residuos de los mismos en diversos alimentos de origen animal, entre ellos la leche y el huevo. Los nitrofuranos se utilizan como aditivos en los piensos para prevenir y tratar las infecciones intestinales. Sin embargo, el uso de nitrofuranos está prohibido en el ganado debido a su carcinogenicidad. Este estudio se diseñó para medir la concentración de residuos de nitrofuranos en la leche y los huevos.
Metodología: Las muestras se seleccionaron en el mercado de Teherán (Irán). La detección se realizó con el método de ensayo inmunoenzimático (ELISA). La preparación de la muestra se realizó según el protocolo del kit.
Resultados: El residuo se detectó en dos muestras de leche a 216,6 y 208,1ng/kg. Sin embargo, no se detectó el residuo del fármaco en ninguna de las muestras de huevos. La ingesta dietética estimada basada en la concentración media de las muestras de leche se calculó en 0,000000031 mg/kg de peso corporal/día.
Conclusión: La IDE calculada no es significativa, pero es necesario evaluar regularmente el nitrofurano residual a partir de los datos de la leche.

Palabras clave: Exposición dietética, metabolito del nitrofurano, residuos, leche, huevo.

Introduction

Eggs are a well-known food that ranks second in quality after breast milk. Eggs are one of the highest quality protein sources and contain almost all of the vitamins (except vitamin C) and minerals needed by the human body. In other words, eggs are the best source of protein that contains essential vitamins and minerals. Milk is one of the most consumed foods in the world, according to the Food and Agriculture Organization of the United Nations[1].

Veterinary medicines for use in animals for specific purposes include the diagnosis, cure, mitigation, management, treatment, or prevention of disease in animals. They can also include modifying any structure or function of an animal's body, such as enhancing reproductive capabilities or other production uses, such as feed efficiency or growth promotion[2]. The presence of drugs or antibiotics residues in food above the maximum acceptable level has been recognized worldwide by various public authorities[3]. Furthermore, Antimicrobial Resistance (AMR) is an increasingly important global health problem, with the potential to render antibiotics unusable and negate medical treatments such as chemotherapy and organ transplant[4] for human concern, AMR in food of animal origin produces a potential threat to direct toxicity in human (cancers, allergic reactions, etc.), and low levels of antibiotic exposure results in alteration of microflora, and the possible development of resistance[5,6].

One of the drugs is nitrofuran, which is widely used as a veterinary drug or food additive in animal husbandry, especially for treating gastrointestinal infections in cattle, poultry, fish, and shrimp. It is an effective antibiotic for the treatment of bacteria and parasites[7]. Its low price and high efficiency are widely used[8].

Among the major compounds to be monitored, some zero-tolerance substances, like nitrofurans (NFs), are prohibited globally[9]. Nitrofurans and their metabolites are suspected of possessing carcinogenic and mutagenic potency[10]. This antibiotic has been used but has been banned in many countries due to its carcinogenicity[11]. Nitrofurans, including nitrofurazone (NFZ), nitrofurantoin (NFT), furaltadone (FTD), and furazolidone (FZD), are a class of synthetic broad-spectrum antibiotics that have been widely used in aquaculture and poultry farming to prevent and treat infections caused by Salmonella spp. and *Escherichia coli*. The presence of these residues in foods of animal origin may lead to adverse reactions in humans, including their carcinogenic, mutagenic and teratogenic effects[12]. In addition, the residues of this drug remain during various cooking processes such as frying, grilling, and microwave[13].

To confirm food safety, it is necessary to regularly check the residue of this drug in samples of animal origin. This article surveyed the number of nitrofurans in milk and eggs supplied in supermarkets in Tehran.

Materials and methods

Samples of eggs and milk were collected in Tehran's north, east, west, and south. According to the specified formula, 81 samples were collected.

Sample preparation for milk samples

Milk samples were transferred into a centrifugal glass vial and added Carrez I and Carrez II. Then, the samples were mixed and centrifuged. The supernatant was mixed with distilled water and HCl, and 2-Nitrobenzoic aldehyde (in DMSO) by shaking vigorously. The homogeneous sample was mixed with distilled water, HCL, and 2-nitrobenzaldehyde. Then, the samples were incubated 3 hours at 50°C and mixed 0.1 M K2HPO4, 0.4 ml of 1 M NaOH, and 5 ml of aniline acetate. The top layer was dried, and one milliliter of hexane and one milliliter of buffer were added to the vials and centrifuged for three minutes at 3000 rpm. The top layer for were used testing

Sample preparation for egg samples

1 g of a homogeneous sample was mixed with 3.9 ml of distilled water, HCl, and 200 ml of 10 mM 2-nitrobenzaldehyde in dimethyl sulfoxide. The samples were incubated for 3 hours at 50°C or 37°C overnight. Then, 5 ml of 0.1 M K2HPOA, 0.4 ml of 1 M NaOH, and 5 ml of ethyl acetate were added to the samples and centrifuged at room temperature for 3 minutes at 3000 rpm. The top layer was dried, and one milliliter of hexane and one milliliter of buffer were added to the vials and centrifuged for three minutes at 3000 rpm. The top layer for were used testing.

Nitrofuran Measurement

Fifty microliters of each standard and each sample are added to the houses with two repetitions. Then, 50 microliters of the conjugate solution were added, and 50 µl of antibody solution was added to each house, shaken gently, and placed at room temperature for one hour. The houses were washed three times with a wash buffer. Then add 100 microliters of dye substrate to each house and incubate for 15 minutes in the room. Finally, add 100 microliters of stopping solution and read to the spectrophotometer after 30 minutes at a wavelength of 450 nm.

Result and discussion

The concentration of nitrofuran in milk and egg samples

Figure 1 illustrates the calibration curve with six different standard concentrations (0, 25. 50,100,200ng/ml). The use of ELISA in the measurement of residual nitrofurans is a valid method and its validity has already been measured. A correlation has been obtained between the results obtained from ELISA and the LC-MS/MS method[14]. ELISA method is frequently used to

Figure 1: Standard curves of nitrofuran (0, 25. 50,100,200ng/ml).

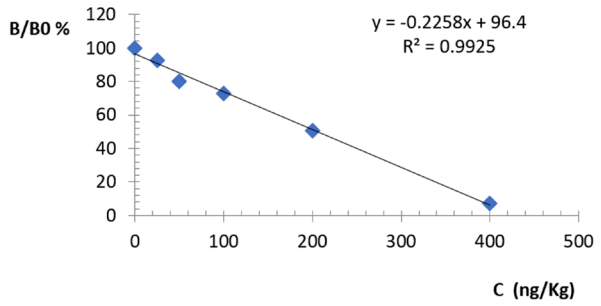

Table I: Nitrofuran metabolite detected (ng/kg) in milk and egg samples.

Product type	Mean ± SD(ng/kg)	EDI (mg/kg bw/day)
Milk	10.6±46.8	0.000000031
Egg	ND	-

evaluate nitrofuran antibiotic residues by government and research centers[15]. The remainder of this antibiotic was not isolated in egg samples but was detected in two milk samples with 216.6 and 208.1 ng/Kg (**Table I**). Cases of illegal or accidental use have been repeatedly recorded in veterinary medicine[16].

The drug is banned in the European Union, Switzerland, the USA, and China. An important global issue is the presence of drug residues in foods of animal origin[17]. Nitrofurans are frequently used in veterinary medicine due to their cheapness, availability, and efficiency[16]. Some foods are not allowed to enter the EU due to nitrofuran antibiotic residues[18]. The maximum residue limit was not set for it and its metabolites[15]. However, it is used in some Asian countries[19]. In previous studies, the residue of this antibiotic has been isolated in shrimp samples in Bangladesh and Irish samples[20]. In a study of animal products in Ireland, 1.8% of the samples had antibiotic residue[21]. Furthermore, it is allowed in animal food and water in Argentina[22].

One of the chemicals that cause problems in the trade of food of animal origin is the residual antibiotic nitrofuran, so these antibiotics should be evaluated regularly[23]. Between 2002 and 2003, the European nitrofuran crisis arose because chicken meat samples imported into Europe contained nitrofuran antibiotic residues[24].

Dietary survey of nitrofuran residue in milk and egg

The per capita consumption of milk in the country is about 80 kg. EDI was estimated using this formula:

$$EDI \text{ oral} = Ci \times Cc / \text{BW}$$

Ci: For the mean concentration of nitrofuran residue in milk (mg/kg) and Cc: The average daily consumption of milk (kg /person/day) and BW: (bodyweight).

Due to the ban on using this antibiotic, no ADI has been established for this antibiotic at the moment, but before 1992, the ADI of Furazolidine metabolite was set at 0004 mg/kg BW. The amount calculated in this study was 0.000000031 mg/Kg BW/day, much less than the ADI of furazolidone. The European Union has adopted a zero-tolerance for both antibiotics and metabolites[22]. Besides the chemical contamination of food samples[25] which was described nitrofurans in the present survey, microbial contaminations were also reported as dangerous issues in food samples[26-31]. Thus, proper food monitoring should perform to avoid their occurrence in food chain.

Conclusion

A total of 81 Samples of egg and milk from markets of Tehran with various commercial brands were collected and analyzed to detect the content of nitrofuran. This is the first report of the detection of nitrofuran metabolites in samples of eggs and milk. The residue of this antibiotic was not detected in all egg samples and most milk samples. In this study, EDI was also calculated for milk samples much lower than ADI previously approved for Furazolidine metabolite.

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Interests conflict

The researchers declare that they have no conflict of interest.

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