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Nitrofuran Metabolite 3-amino-2-oxazolidinone Residues in Chicken Liver: A Screening Study

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ABSTRACT

Enzyme Linked Immunosorbent Assay (ELISA) method has been implemented to screen the residues of nitrofuran metabolite AOZ in chicken livers obtained from local markets in Bursa province of Turkey. For this purpose, ELISA kit specific for this metabolite was used. ELISA screening demonstrated the presence of AOZ residues in 11 of 90 chicken liver samples, with a positive rate of 12.2%. The residual level of nitrofuran metabolite AOZ in chicken livers ranged from 103.8 to 1027.8 ng kg^{-1}, having a mean of 212.2 ng kg^{-1}. Present results showed that the use of these compounds in broiler has the potential and public awareness should be raised to prevent the usage of AOZ in livestock production.

Key words: Antibiotic residue, nitrofuran metabolites, chicken, ELISA

INTRODUCTION

Furazolidone, furaltadone, nitrofurazone and nitrofurantion are veterinary drugs that belong to the nitrofuran group (Finzi *et al*., 2005; Stolker and Brinkman, 2005). Nitrofurans were commonly employed as feed additives for growth promotion and mainly used for livestock (i.e., poultry, swine and cattle), aquaculture (i.e., fish and shrimp) and bee colonies in the prophylactic and therapeutic treatment of bacterial and protozoan infections such as gastrointestinal enteritis caused by *Escherichia coli* and *Salmonella* spp. (Rodziewicz, 2008; Vass *et al*., 2008).

Nitrofurans form protein-bound metabolites (isolation of bound residue) which are 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) for furaltadone, 1-amino hydantoin (AHD) for nitrofurantoin, semicarbazide (SEM) for nitrofurazone and 3-amino-2-oxazolidinone (AOZ) for furazolidone (Verdon *et al*., 2007). The nitrofurans are quickly metabolized and are not detected after few hours from their administration. However, nitrofuran metabolites remain during months as residues bound to tissue proteins (Finzi *et al*., 2005). Thus, methods for detecting residues of nitrofurans by measuring the parent drugs are inappropriate. Methods of analysis for identifying the use of furazolidone in animals have used AOZ as the target analyte (Conneely *et al*., 2003).

For nitrofuran metabolites the maximum residue limit (MRL) could not be set in food-producing animals. The European Commission Decision 2003/181/EC established the minimum required performance level (MRPL) at 1 μg kg^{-1} for each nitrofuran metabolite in poultry meat and aquaculture products (European Commission, 2003).

If nitrofuran remains in food, it causes mutagenesis, carcinogenicity and teratogenesis (Tsai *et al*., 2009; Verdon *et al*., 2007). Due to the toxicological hazard for human consumers provoked by these drugs, the European Union (EU) prohibited the use of nitrofuran antibiotics in food-producing animals (CEC, 1990). The use of nitrofurans for livestock has also been banned in Turkey and other countries such as Australia, USA, Thailand and Brazil (Vass *et al*., 2008).

In this survey, we aimed to screen the presence and level of nitrofuran AOZ residues in chicken liver samples on retail sale in Bursa province by ELISA technique.

MATERIALS AND METHODS

Samples: During December 2008 and August 2009, a total of 90 chicken liver samples were purchased from different supermarkets and retail stores in Bursa, Turkey. All of the samples were maintained frozen (-20°C) until use.

Detection of nitrofuran metabolite AOZ with ELISA

Sample preparation: A 50 mL centrifuge tube was weighed 1 g of blended chicken liver and added 3.9 mL of deionized water, 0.5 mL of 1 M HCl and 100 μL 10 mM 2-nitrobenzaldehyde in dimethyl sulfoxide. Each tube was incubated overnight (approximately 16 h) at 37°C and added 5 mL of 0.1 M K_2HPO_4, 0.4 mL of 1 M NaOH and 5 mL of ethyl acetate. After shaking vigorously for 1 min, these tubes were centrifuged 10 min at 3000 g to separate layers. 2.5 mL of the ethyl acetate layer was transferred into a new centrifuge tube and dried under reduced pressure in a rotary evaporator. The residue was dissolved in 1 mL n-hexane and mixed with 1 mL of sample buffer. Following centrifugation 10 min at 3000 g, 50 μL of upper aqueous phase was used for ELISA analysis.

ELISA assay: For the quantitative detection of nitrofuran metabolite AOZ in chicken liver samples, Ridascreen Nitrofuran (AOZ) test kit (R3701, R-biopharm, Germany) was used. The detection limit of the test was 100 ng kg^{-1} and recovery rates were >80 for all samples. ELISA technique was performed as suggested by the kit manual.

Briefly, 0-negative control, 25, 50, 100, 200 and 400 ng kg^{-1} of each standard solution and 50 μL of the previously prepared samples were added to microtiter wells, sample and standard positions were recorded. Then 50 μL of the enzyme conjugate and 50 μL of the antibody solution were added to each well and incubated for 1 h at room temperature. At the end of incubation, the micro wells were washed three times with 250 μL of washing solution and the liquid in them was poured out. One hundred microliter of substrate/chromogen was added to each well and they were gently shaken. The wells were incubated for 15 min at room temperature in the dark. Finally, 100 μL of the stop solution (1N H_2SO_4) were added to each well and the absorbance at a wavelength of 450 nm was measured with ELISA plate reader (Rayto RT-2100C, Rayto Corporation, Shenzhen, China). The data obtained from the standards and samples were evaluated using a special software RIDAWIN (R-biopharm, Darmstadt, Germany).

RESULTS AND DISCUSSION

In this study, we report the results of the determination of nitrofuran AOZ residues using ELISA in chicken liver samples (n = 90) collected from different local retailers in Bursa province. The results of the ELISA screening of samples are presented in Table 1 and 2 show the distribution of nitrofuran metabolite AOZ in the samples. Among a total of 90 chicken liver samples, the incidence of AOZ was 12% within the range of 103.8- 1027.8 ng kg^{-1}.

Table 1: Nitrofuran AOZ residue determination of chicken livers by ELISA

Statistics	Values
No. of samples	90
No. of positive samples (%)	11 (12.2)
Mean±SD (ng kg^{-1})	212.2±271.5
Minimum (ng kg^{-1})	103.8
Maximum (ng kg^{-1})	1027.8

Table 2: Frequency of nitrofuran metabolite AOZ residues in chicken liver

Nitrofuran AOZ levels (ng kg^{-1})	No. of samples	Frequency (%)
<100[1]	79	87.8
100-115	2	2.2
115-130	4	4.4
130-145	3	3.3
>145	2	2.2

[1]The limit of detection (LOD) for ELISA

The nitrofurans are antimicrobial drugs that have been widely used as veterinary therapeutics or feed additives for treating bacterial diseases in food producing animals (Verdon *et al*., 2007). However, the use of nitrofurans for these purposes in livestock production has been banned in the EU (CEC, 1990) due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Vass *et al*., 2008).

Apart from its long term stabihty in tissue, 3- amino-2-oxazolidinone (AOZ), belong to the group of nitrofuran antibacterial drugs, is not degraded by common cooking techniques (Franek *et al*., 2006) and thus it is essential to monitore and to detect metabolite AOZ residues in edible animal tissues. The present study was performed for screening by ELISA of the metabolite AOZ, in chicken livers at the retail level in Bursa province. As shown in Table 1, 11 (12%) of the samples tested were found to contain AOZ residues. The levels of AOZ ranged from 103.8 to 1027.8 ng kg^{-1}, with a mean level of 212.2 ng kg^{-1}. AOZ was not found in the remaining 79 (87.8%) samples, its levels were below of the detection limit (LOD, 100 ng kg^{-1}). From the data available, it appears that nitrofuran antibiotics are still used in poultry industry as growth promoters and prophylactic agents despite strict legislation banning its use for livestock production.

The presence of the residues of nitrofuran metabohte AOZ in animal origin foods has been also reported by McCracken and Kennedy (1997) suggested that using LC-MS/MS seventeen of one hundred pork samples analysed contained the residues of this drug. O'Keeffe *et al*. (2004) also reported that residues of nitrofuran metabolites by LC-MS/MS were confirmed in 12 of 1500 pork samples of which two contained AOZ at concentrations of 0.3 and 3.0 μg kg^{-1}. A similar observation has been made by Mottier *et al*. (2005) showing by LC-MS/MS method AOZ was detected 15% of the meat based products. In a study performed by Tsai *et al*. (2009), the content of nitrofuran metabohte AOZ in *Tilapia* tissue was determined using both the ELISA and LC-MS/MS methods.

Although, immunoassay techniques are very sensitive, the potential lack of specificity is a drawback since other compounds of similar chemical structures present cross-reactivities (CEC, 1990). The EU have recommended that where possible, some type of mass spectrometry should be used in order to increase specificity (European Commission, 2003). From this point of view, we further analyzed the some ELISA positive and negative results for confirmation with

LC-MS/MS validated according to the criteria of the European Commission Decision 2002/657/EC (European Community, 1996) and successfully applied the method for confirmation of these residues.

CONCLUSION

The results found in this study show the occurrence of the residues related to nitrofuran metabolite AOZ in chicken liver samples from Bursa province and the potential for illegal use of this drug in poultry production. To reduce the risk of potentially harmful drug residues for consumers' safety, it is necessary to control the use of such metabolites in livestock production and to follow up the periodic analysis of foodstuffs for the residues.

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