PAPER

THE EFFECT OF COOKING AND STORAGE ON FLORFENICOL AND FLORFENICOL AMINE RESIDUES IN EGGS

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ABSTRACT

The aim of this study was to evaluate the effects of storage conditions (room temperature, refrigerator) and cooking methods (frying, boiling) on florfenicol (FF) and florfenicol amine (FFA) residue levels in eggs. Without any significant difference between storage conditions at 20˚C and +4˚C, residue levels decreased within days, but were still present on day 28. Frying and boiling for 1 and 5 min yielded similar results to the storage conditions just described; there was a significant decrease in residue levels, but still not enough for decomposing. These findings indicate that FF and FFA residues are heat-labile.

- Keywords: cooking, egg, florfenicol, florfenicol amine, residue, storage -

INTRODUCTION

When veterinary drugs are administered to farm animals, either therapeutically or to promote growth, residues remain in their meat, milk or eggs if proper precautions are not followed (BOTSOGLOU and FLETOURIS, 2001). Antibiotics play an important role among such drugs. In addition to their positive effects, they can also cause health problems, including drug hypersensitivity (PAIGE *et al.*, 1999; DONOGHUE, 2003). Antibiotics not only threaten food safety, but also cause the development of some resistant bacterial strains from among sensitive bacteria even when used at moderate doses for long periods of time (PAIGE *et al.*, 1999; FILAZI *et al.*, 2005).

Testing for drug residues are ordinarily performed on raw products. Almost no edible animal products or byproducts are consumed raw, but require some type of processing or cooking, such as frying, boiling, or roasting, before consumption. These processes can cause denaturation of proteins, elevation of temperature, loss of water and fat, and pH variations that can eventually result in alteration to the concentration, chemical nature, chemical reactions, and solubility of drug residues in a particular food item. Many drugs are chemically unstable to varying degrees, and therefore may undergo degradation during storage, cooking or processing in consumable foods (BOTSOGLOU and FLETOURIS, 2001).

In general, the temperatures achieved during cooking are assumed to degrade antibiotic residues in food; however, ordinary cooking procedures are unreliable for degrading or inactivating several commonly used veterinary drugs. Earlier studies have indicated that sulfamethazine (ROSE *et al.*, 1995; PAPAPANAGIOTOU *et al.*, 2005) chloramphenicol (BOTSOGLOU and FLETOURIS, 2001), streptomycin (INGLIS and KATZ, 1978; O'BRIEN *et al.*, 1980), neomycin (KATZ and LEVIN, 1978), gentamicin (SIRELI *et al.*, 2006), fluoroquinolones (BAYDAN *et al.*, 2000a, b; BAYDAN *et al.*, 2002), penicillin G (NOUWS and ZIV, 1976; BOISON *et al.*, 1992), nitrofurantoin (COOPER and KENNEDY, 2007), oxacillin, clindamycin, novobiocin, trimethoprim, vancomycin, and azlocillin are heat-stable (TRAUB and LEONHARD, 1995), whereas oxytetracycline (KITTS *et al.*, 1992) and amphenicols (FRANJE *et al.*, 2010) heat-labile. On the other hand, several β-lactams including ampicillin and amoxicillin are partially heat-labile (TRAUB and LEONHARD, 1995). Antibiotics of the same class were reported to vary in heat stability according to the type of matrix and heating treatment involved (KITTS *et al.*, 1992; ROSE *et al.*, 1996; FRANJE *et al.*, 2010). As such, the effect of different matrices on the stability of every veterinary drug should be investigated.

Although most edible animal products are consumed after cooking or some type of processing, for the licensing of veterinary drugs research concerning the effects of storage and cooking of the drugs on different matrices are lacking. Most data on drug residues in edible animal products and government regulation concern raw products. It is therefore essential to determine the effect of processing on all veterinary drugs when assessing human exposure to drug residues in animal food products (IBRAHIM and MOATS, 1994; MOATS, 1988; MOATS 1999; BOTSOGLOU and FLETOURIS, 2001).

Florfenicol (FF) is a wide-spectrum, synthetic antibacterial that is structurally related to D(–) threo-chloramphenicol; however, FF differs from chloramphenicol in that FF contains a *p*-methyl sulfonyl group instead of a *p*-nitro group and it contains a fluorine atom instead of a hydroxyl group in the terminal primary alcohol group (EMEA, 1999). FF has not been approved for use in laying hens; however it is used in cattle, swine, poultry, and fish (EMEA, 2000).

FF is metabolized into florfenicol amine (FFA), florfenicol oxamic acid, florfenicol alcohol, and mono-chloroflorfenicol in animals. FFA is the longest-lived metabolite in the bovine liver; therefore, FFA can be used as a marker for the calculation of withdrawal time (ANADON *et al.*, 2008; XIE *et al.*, 2011).

In light of the apparent advantages over chloramfenicol and its availability as an additive, the potential for off label use of FF is high. Due to its broad spectrum antibacterial activity, ready availability and low cost, it remains a possibility that FF residues will continue to be found in such animal food products as eggs. For example, Xie *et al.* (2011) analyzed 50 egg samples obtained from a local supermarket in China and reported 19 ppb of FF and 36 ppb of FFA in only 1 egg. FILAZI *et al.* (2014) reported that the concentration of FF and FFA in eggs were 0.1%, 0.08% respectively regardless of the route of administration.

Data on the heat stability of FF is essential for food safety; however, the literature contains few data regarding its heat-stability during cooking. Under environmental conditions FF is stable at 25 °C, yet photodegradation occurs at varying rates in water under various lighting conditions (GE *et al*, 2009). FF was shown to rapidly degrade to FFA in the deep sediment of marine environments via biodegradation (HEKTOEN *et al*, 1995). A few studies on the residue of FF and FFA in eggs have been published (XIE *et al.* 2011; FILAZI *et al.*, 2014); but the data are insufficient. FRANJE *et al.* (2010) reported that amphenicols exhibit differential behavior in terms of heat-induced degradation in solutions and protein matrices. Although the level of amphenicol degradation in soybean sauce and meat was high, heating may generate product with antimicrobial activity; therefore, heating amphenicol residues in food cannot always be considered safe. FF is the most commonly used veterinary antimicrobial agent in Turkey, particularly so due to its illegally use in laying hens. Nonetheless,

few studies have examined FF residue levels in eggs. An earlier study reported that FF and FFA were detected in the eggs of hens administered with FF (FILAZI *et al.*, 2014)

Chicken eggs are widely used in the preparation of many types of food, including many baked goods. Some of the most common preparation methods include fried in oil, hard-boiled, soft-boiled and omelets. Data regarding FF and its main metabolites in cooked and stored eggs are lacking. As such, the aim of the present study was to determine the effects of different storage conditions (room temperature and refrigeration) on FF residue levels in eggs stored up to 28 days and to determine the effect the different cooking methods (frying and boiling) on FF and FFA residue levels.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Ankara University Ethics Committee (2007-15-45). The study included 50 ISA Brown laying hens aged 48 weeks and weighing 1.9-2.4 kg. The hens were housed individually in fiber cages (30x35x45 cm), in a ventilated room maintained at 20°C under 14 h day light condition. The hens received standard commercial layer mash (120 g/d) and water ad libitum. The hens were fed for 1 week and their eggs were collected for preliminary analysis to determine if they were analyte-free.

Trials

A veterinary drug containing 300 mg of FF in 1 mL was used (Mediflor 30% Oral Solution, Medicavet Company, Turkey) for the clinical trials. FF was administered at a dose of 20 mg/kg/day via gavage for 3 days to the 50 laying hens, and then their eggs were collected daily thereafter. The effect of storage procedures on the residues was determined on the first day using 44 eggs. The effects of cooking procedures were determined on the second day using 32 eggs. In all, 20 of the eggs collected on the first day were kept at 4°C in a refrigerator, and 20 were kept at 15-20°C (room temperature). In addition, 4 eggs were analyzed on day 4, 7, 14, 21, 28 of storage to determine FF and FFA residue levels. Lastly, 4 uncooked eggs collected on day 1 were analyzed as a control group; of the eggs collected on day 2, 8 uncooked, 8 fried in oil, 8 undercooked (1 min in boiling water) and 8 overcooked (5 min in boiling water) were then analyzed.

Sample preparation and analysis

FF and FFA were extracted from homogenized eggs via phosphate buffer (pH:7) and ethyl acetate. Following purification, the samples underwent high-performance liquid chromatography (HPLC) using a photodiode array detector (PDA) and C18 column; the method was validated according to ICH guidelines, as described elsewhere (FILAZI *et al.*, 2014). According this method, limits of detection and of quantitation values were 1.94 and 6.45 ppb for FF, respectively, and 0.48 and 1.58 ppb for FFA, respectively. Relative standard deviation values of intra-day and inter-day variation below 11% also confirmed the usefulness of the method for analysing FF and FFA in eggs.

STATISTICAL ANALYSIS

Variance analysis was performed with all data and a multiple range test was used to determine the differences between groups. All analyses were performed using SPSS v. 17.0 for Windows.

RESULTS AND CONCLUSION

The effects of different storage temperatures and durations on FF and FFA residue levels in

Table 1 - Mean±SD* concentration (in ppb) of florfenicol and florfenicol amine residues in eggs stored at room temperature (15-20°C) and in a refrigerator (+4°C).

Days	Florfenicol		Florfenicol amine	
(n=4)	Room temperature (15-20°C)	Refrigerator (+4°C)	Room temperature (15-20°C)	Refrigerator (+4°C)
0	290.65±11.02^{a}	290.65±11.02^{a}	91.79±6.77^{a}	91.79±6.77^{a}
4	151.24±10.69^{b}	167.43±8.18^{b}	58.26±5.98^{b}	58.61±5.85^{b}
7	79.65±9.43^{cx}	105.10±4.25^{cy}	28.95±5.03^{c}	35.40±2.33^{c}
14	68.23±8.74^{dx}	87.84±5.01^{dy}	20.52±3.92^{d}	24.37±1.20^{d}
21	29.43±4.91^{ex}	61.82±2.11^{ey}	10.42±1.54^{e}	8.54±1.04^{e}
28	18.57±3.48^{f}	22.14±0.03^{f}	6.74±0.79^{f}	7.06±1.21^{f}

*SD: Standard Deviation.
abcdef: Differences between values with different letters in the same columns are significant (P<0.05).
xy: Differences between values with different letters in the same rows are significant (P<0.05).

Table 2 - Mean±SD*, quantity of florfenicol and florfenicol amine residues (in ppb) after different cooking methods.

Residues (n=8)	Raw	Fried	Undercooked (1 minute)	Overcooked (5 minutes)
Florfenicol	265.45±13.67[a]	56.51±9.68[b]	35.67±4.57[c]	5.68±1.17[d]
Florfenicol amine	110.31±12.73[a]	19.77±4.71[b]	10.20±1.72[c]	4.57±0.92[d]

*SD: Standard Deviation.
abcd: Differences between values with different letters in the same row are significant (P<0.05).

eggs are shown in Table 1. Both FF and FFA amine residue levels in eggs were observed on day 28, though their levels had decreased significantly (P<0.05). HEKTOEN *et al.* (1995) reported that FF rapidly depurated in the sediment of marine environments and that its metabolite (FFA) was isolated from the sediment. This finding suggests that FF is degraded to FFA in the sediment via metabolization or leaching; however, the present study FF residues in eggs following storage for 28 day at room temperature and in a refrigerator were observed. FF residue levels in eggs were higher than FFA residue levels in the present study, which indicates that the in vitro degradation of FF might occur at a very low level or that it differs from its biological degradation. Further research would be required to understand the effect of storing on the FF and FFA residues in the eggs.

FRANJE *et al.* (2010) studied the heat stability of amphenicols in chicken meat and reported that 5-min heating of amphenicols in water in a microwave oven generated a comparable percentage of degradation as did boiling in a water bath for 30 min 1 h; FF produced thiamphenicol (TAP) as a product of its breakdown, but not FFA. It was reported that although a higher level of degradation of amphenicols was observed in soybean sauce, heating treatment might still generate product with antimicrobial activity (FF to TAP) and as such, heating amphenicol residues in food cannot always be safe.

FF was reported to be hydrolytically stable and to have a hydrolysis half-life ≥ 1 year at 25°C in natural waters (HAYES *et al.*, 2003; POULIQUEN *et al.*, 2007; GE *et al.*, 2009). GE *et al.* (2009) performed photodegradation experiments on TAP and FF in aqueous solutions under irradiation from different light sources. They reported that under UV-Vis irradiation (λ>200 nm) photodegradation in seawater was fastest, followed by pure water and freshwater, whereas under solar or simulated sunlight (λ > 290 nm), photodegradation occurred only in freshwater. Under UV-Vis irradiation, Cl- (dominant sea water constituent) was observed to promote singlet oxygen formation and accelerated the photodegradation of phenicols, whereas phenicols did not photolyze under simulated solar irradiation, irrespective of the presence of Cl^-.

In contrast, HAYES *et al.* (2003) reported that FF was stable under a range of simulated field conditions, including various pipe materials and conditions of hard and soft and chlorinated or non-chlorinated water at low or high pH; therefore not only Cl^- but also some other minerals might effect the stability of FF.

The effects of different cooking procedures on FF and FFA residues in eggs observed in the present study are shown in Table 2. Even though, none of the cooking methods completely destroyed FF or FFA residues in eggs, there was a significant decrease in the level of detectable FF and FFA residues (P<0.05). Concentrations of both analytes were reduced by 78%-97% via frying and boiling. These findings suggest that FF and FFA heat labile in eggs, which indicates that both do not bind to proteins in eggs with high affinity. FRANJE *et al.* (2010) reported that amphenicol degradation was apparent following as little as 30 min of heating and that it was correlated with the length of heating, implying that as cooking time increased the degree of residual drug present in samples decreased; as such, it could be assumed that there was a strong correlation between the decrease in FF and FFA concentrations in observed eggs during different cooking methods and the duration of cooking (P<0.05, Table 2). SHAKILA *et al.* (2006) studied the stability of chloramphenicol (CHP) residues in white shrimp (*Penaeus indicus*) subjected to cooking (100 °C) for 10, 20 and 30 min as well as retorting (121°C) for 10 and 15 min, based on a microbial assay method using *Photobacterium leiognathi* as the test organism. They reported that the loss of CHP increased as temperature and duration of heating increased, where the drug could be completely destroyed. On the other hand BOTSOGLU and FLEUTORIS (2010) reported that CHP was quite stable under heating conditions when added to water or milk; after 2 h of boiling, it was decreased by ≤8%. These findings indicate that the heat stability of amphenicols is matrix dependent, where results from different matrices could not be attributed to eggs when interpreting.

Even though, FF is not approved for use in laying hens, its off label use for severe indications can result in antibiotic residues in eggs that both farmers and consumers should be informed about. As such, drug withdrawal periods should be extended prior to poultry slaughter

or egg distribution to avoid antimicrobial resistance. Thermal treatments may reduce the concentration of veterinary drug residues in foods and thereby might reduce the pharmacological and/or toxic effects of these compounds. (HSIEH *et al.*, 2011). In the current study, FF and FFA were observed to be heat labile in chicken eggs, the level of which depended on cooking method and duration.

The findings show that FF and FFA residue levels in eggs from treated laying hens were not completely eliminated via cooking or of up to 28 d; however, cooking did significantly decreased the level of the drug in eggs.

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The authors declare no competing financial interest.

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