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Full Length Research Paper

Effect of roasting, boiling and microwaving cooking methods on Enrofloxacin residues in edible tissues of broiler

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The purpose of this study was to determine the effects of different cooking processes like boiling, roasting and microwaving on Enrofloxacin residues in chicken muscle, liver and gizzard tissues of broiler chickens. Each of the chicks was fed by water and food with 0.05% of Enrofloxacin in their drinking water for 5 consecutive days. Then, three locations were sampled aseptically from each carcasses: breast muscle; liver and gizzard. Enrofloxacin residue was analyzed using microbial inhibition method by plates seeded with *Escherichia coli*. After doing different phases of the test on raw samples, the positive raw samples were cooked by various cooking procedures and the cooked samples were surveyed with similar method again for the presence of residue. The results showed the reduction in concentration of Enrofloxacin residue after different cooking processes. The most reduced residue in cooked meat and gizzard samples related to boiling process and the cooked liver samples was the roasting process. The highest detectable amount of residue belonged to microwaving process in all cooked samples. Regarding to the results of this study, it was concluded that, cooking processes cannot annihilate total amounts of this drug and it can only decrease its amounts. Also, most of the residue in boiling process was excreted from tissue into cooking fluid.

Key words: Cooking, Enrofloxacin, residue, poultry, edible, tissue.

INTRODUCTION

The quinolones are a group of synthetic antimicrobial agents that have a wide spectrum of action and high efficacy against various bacterial infections, especially Gram-negative bacteria but lesser against gram-positive cocci (Xu et al., 2006; Salehzadeh et al., 2007). Also, they have been recommended for the treatment of urinary tract and enteric infections in humans. The anti-bacterial activity of quinolones is based on the inhibition of DNA-gyrase which leads to an unstable condensation of the DNA configuration of the bacterial DNA molecule during cell division (Xu et al., 2006).

Antibiotics are normally administered by veterinarians for treatment, prevention of infection disease in farm animals and it is an important measure when raising animal under intensive husbandry methods production (Maraschiello et al., 2001; Dipeolu et al., 2002). In addition, they are routinely used at sub-therapeutic levels as animal feed additives for their growth promoting properties (Okerman et al., 1998a). Existence of antibiotic residues in food stuff can pose hazards to human health. Among them are sensitivity to antibiotics, allergic reactions and imbalance of intestinal microflora, bacterial resistance to antibiotics in microorganisms and losses in

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the food industry (Cunha, 2001; Kirbiš, 2006; Lolo et al., 2006). Also, the high level of use in animals and humans, unnecessary use or use of quinolones with poor activity in developing countries and especially in Iran has been blamed for the rapid development of bacterial resistance to these agents and it has not remarked to withdrawal times of antibiotics in food-producing animal industry. So, quality control of food stuff regarding to antibiotic residues, is necessary (Salehzadeh et al., 2007).

Enrofloxacin is a synthetic fluoroquinolone antimicrobial agent. In veterinary medicine, it is administered orally to turkeys and chickens, for the treatment of infections of the respiratory and alimentary tract. The recommended doses are 10 mg Enrofloxacin/kg bw/day for 3 to 10 days (chickens and turkeys) (EMEA, 1996). Currently, levels of drug residues in raw food (meat and animals products) is regulated. Maximum residue limits (MRL) for the fluoroquinolone, Enrofloxacin, and its metabolite, ciprofloxacin, legally permitted in food under European Union regulations (EEC, 1990). European Union (EU) countries established a maximum residue level (MRL) of 30 ng/g of muscle, liver and kidney for sum of Enrofloxacin and ciprofloxacin (Posyniak et al., 2001; Lolo et al., 2006; Salehzadeh et al., 2007). Enrofloxacin was introduced for veterinary use in Iran in 1990 and has been available since 1991 in pure powder and solution form for addition to poultry drinking water, prophylaxis or treatment of infections due to Gram-negative microorganisms. In Iran, about 20% of antibiotic consumption relate to Enrofloxacine (Salehzadeh et al., 2007).

In detection of antimicrobial residues, microbiological methods are the basis of screening methods for monitoring the presence of veterinary drug residues in foods of animal origin (Hussein, 2004). They are used as the mainstream screening methods for systematic detection of antibiotic residues in food and they determine the presence of antibiotics in the sample and identify the specific antibiotic groups (Aerts et al., 1995; Haasnoot et al., 1999). Screening methods have acceptable false-positive result rates (Korsrud and MacNeil, 1987) and allow detection of a wide spectrum of antibiotics (Aerts et al., 1995; Haasnoot et al., 1999). Their other advantages are the option to analyze a large number of samples simultaneously and the relatively short time needed for preparation of samples as no purification procedures are required. They cannot be used to identify individual antibiotics. A positive result should be confirmed with chemical or physical methods (Ferrini et al., 2006; Kirbiš, 2006). Microbial methods are relatively inexpensive, easy to use, do not require expensive equipment and can be efficiently adopted by laboratory staff. On the basis of other researches, the plate seeded with *Escherichia coli* is suitable for detection of fluoroquinolones residues (Okerman et al., 1998b; Gaudin et al., 2004).

Between 1995 and 1999, Rose and his co-workers demonstrated that residues of a range of veterinary drugs have varying degrees of stability during cooking and, therefore, the cooking influences the level of risk posed by such residues (Rose et al., 1999). Since the most of food producing animals are always cooked before consump-tion and the variations in Enrofloxacin levels in the tissue are dependent on the type of cooking (Lolo et al., 2006), more findings about the effect of cooking on Enrofloxacin residue are needed to accurately determine consumer exposure to this drug.

MATERIALS AND METHODS

Chickens and drug administration

Twenty male broiler chickens (Ross 308) (3 weeks old) were randomly divided into 2 groups; control group and case group; each containing 10 chicks. In order to remove their bodies from the probable antibiotic residue; they were fed by free antibiotic food for around 10 days. Each of the chicks in the case group was fed by water and food with 0.05% of Enrofloxacin in their drinking water for 5 consecutive days and chicks in the control group were fed with similar water and food but without Enrofloxacin for a similar period.

Preparation of samples

After the 5th day of the drug administration, chickens were killed and breast muscles; livers and gizzards were sampled aseptically from each carcass. After notation of sample characteristics; we placed them in sterile polyethylene containers.

Cooking operation

Boiling: A 20 g sample was placed into a strainer, immersed in 10 ml of water bath preheated to 100°C and cooked for the specified time (9 min for liver samples; 24 min for muscle samples and 85 min for gizzard samples), removed and allowed to cool.

Roasting: A 20 g sample was placed on a metal baking tray and cooked to be well done in the center of electric oven (Memmert, Germany) at 200°C for the specified time (25 min for liver samples, 40 min for muscle samples, 60 min for gizzard samples), removed, and allowed to cool. No juices, which came from the samples as they were cooked, were collected. The cooked muscle had a ‘‘well done’’ appearance on the outside.

Microwaving: A 20 g sample was placed on a turned table. The sample was cooked under full power (900 W) for the specified time (3 min for all samples), removed and allowed to cool. No juice was collected.

Test procedure for raw and cooked samples

The test organism that was used in this study is *E. coli* (PTCC 1270) and the used agar Medium was Muller Hinton agar (Quelab, England) and this medium was adjusted to pH = 6 with sodium

hydroxide and acid autoclaved as indicated by the manufacturers. Sterile Petri dishes (diameter 90 mm) were filled with 25 ml of the prepared culture medium then we seeded *E. coli* in plates. Raw sample disks (diameter 2 mm) were put on each plate; also, we put a paper disk for negative control. After all samples were put onto the plates, plates were incubated at 37°C for 24 h. A positive raw sample is indicated by a complete inhibition of growth in an annular zone not less than 2 mm wide around the disc. Less than 2 mm of inhibitory zone indicated negative result (Myllyniemi et al., 2001). Results of inhibition zones diameter was read by digital caliper after incubation of plates.

The positive raw samples were selected for cooking processes (boiling, roasting and microwaving) then we performed the test for cooked samples just like raw samples after complete cooking of them. Also, we placed 0.01 ml of boiling fluid on plates after the boiling process of samples for detection of residues.

Analytical method

Comparison between the mean diameter of inhibition zones around raw and cooked samples analyzed by ANOVA test and SPSS software version 15.

RESULTS

Comparison of the effects of different cooking methods on the mean diameter of inhibition zones (mean ± SE) around raw and cooked samples are shown in Table 1. We saw that all cooking processes can lead to a reduction in diameter of inhibition zones in cooked samples rather than raw samples. Comparison of the effect of each cooking process on the mean inhibition zones diameter (mean ± SE) around different tissues samples are shown in Table 2. All of the tissues had a reduction in their inhibition zone diameter in each cooking method. Only the difference between the mean inhibition zones of boiled muscle and liver was not significant (Table 2).

DISCUSSION

The microbiological detection methods are used to establish whether and where antimicrobial residues accumulate in the tissues of commercial animal farming. They are essentially a qualitative screening test, which detects any tissues substance with the property of bacterial inhibition. The advantages of these tests are quite simple, inexpensive, sensitive, reliable, and they do not have need for high skill of operator. In the microbial test, observation of inhibition zones is possible when antibiotics residue is above MRL because this test cannot detect amounts of residues below or around allowable amounts.

According to the results of our study, maximum mean inhibitory zone in all cooked samples regarding to microwaving process and minimum inhibitory zone related to boiled samples in the cooked muscle and gizzard samples and roasting process in the cooked liver samples (Table 1). The cooked muscle and liver have the most and lowest detectable remaining residue in boiling fluid, respectively (Table 2). These results proved that, most of the residues were excreted from tissue to cooking fluid in the boiling process. The most reduction of Enrofloxacin residue in cooked muscle and gizzard samples related to boiling and roasting processes for cooked liver samples and the highest detectable amount of residues belonged to microwaving process in all cooked samples (Table 1). Difference between the residues of raw and cooked muscle samples and difference between the residues in various cooking processes from viewpoint of significance are shown in Table 1 ($P<0.01$). Also, the differences between the residues of different tissues in each cooking process was significant except between the boiled muscle and liver samples ($P<0.01$) (Table 2).

Based on a research about the effect of different cooking processes (microwaving, roasting, boiling, grilling and frying) on Enrofloxacin residues in the breast and whole leg of chicken, the scientists mentioned that the extraction of residues took place when the chicken pieces were boiled at 100°C for 10 min or microwaved at 800 W for 3.5 min and there was a reduction in concentration and the lost amount of residue from the tissue found in water or exudates and the amount of residue increased with roasting at 200°C for 10 min and grilling for 10 min because the lower moisture content of the treated piece caused an apparent concentration of the quinolone residue (Lolo et al., 2006). The results of boiling and microwaving in this research confirm the findings of our study about the decrease of Enrofloxacin activity, after cooking but there is no consistency with our results about roasting processes and we can relate this variation to the difference in roasting time in our research that it was 40 min for muscle samples for complete cooking and another reason can be related to the detection method that we used; qualitative microbiological method instead of HPLC (High Performance Liquid Chromatography) method as a quantitative method.

The results of our study are consistent with other studies about the fate of drug residues. According to a study on the stability of antibiotics in a pork meat-kidney-liver mixture after the sterilization step (134°C for 20 min), it was proven that, the mean remaining activity of Enrofloxacin residue reduced to 68% after cooking (Van Egmond et al., 2000) or in a study on Norfloxacin residue in the liver and muscle samples of poultry in the eastern province of Saudi Arabia, from all positive raw samples with concentration of Norfloxacin residue above MRL, 40.5% of muscle samples and 72.1% of liver samples had residue above MRL after cooking in water at 100°C for 20 min (Al-Mustafa and Al-Ghamdi, 2000).

Table 1. Comparison of mean inhibition zones diameter (mean±SE) between raw and cooked samples in different cooking procedures.

	Muscle	Liver	Gizzard
Raw	24.6 ±0.99^{c}	19.3± 2.24^{b}	20.2 ± 0.55^{c}
Boiled	7.1 ± 0.87^{a}	9.3 ±1.30^{a}	6.30 ± 0.94^{a}
Boiling fluid	14.7 ±1.02^{b}	12 ±2.99^{ab}	13.6 ± 0.98^{b}
Microwaved	16.3 ±1.20^{b}	11.9 ±3.27^{ab}	16.9 ±1.13^{bc}
Roasted	7.6 ± 0.31^{a}	5.9 ±0.81^{a}	6.80 ± 0.25^{a}

^{a} ,^{b} and ^{c} : Differences between the means that have common letters are significant (P<0.01).

Table 2. Comparison of mean inhibition zones diameter (mean±SE) in liver, muscle and gizzard samples in each cooking method.

	Boiled	Boiling fluid	Microwaved	Roasted
Muscle	7.1 ± 0.87^{b}	14.7 ±1.02^{a}	16.3 ±1.20^{a}	7.6± 0.31^{a}
Liver	9.3 ±1.30^{a}	12 ±2.99^{a}	11.9 ±3.27^{a}	5.9 ± 0.81^{a}
Gizzard	6.30 ± 0.94^{a}	13.6 ± 0.98^{a}	16.9 ±1.13^{a}	6.8± 0.25^{a}

^{a} and ^{b} : Differences between the means that have common letters are significant(P<0.01).

Ciprofloxacin has been described as the main metabolite of Enrofloxacin with extensive metabolism in chickens but only small amounts or undetectable levels of Ciprofloxacin can be found in samples (Lolo et al., 2006).

According to the results of this paper and findings of another research about effects of different cooking procedures on antibiotic residue in food stuff, we can conclude that cooking processes cannot annihilate the total amounts of this drug but it can only decrease their amounts and most of the residues in boiling process are excreted from tissue to cooking fluid during the boiling process. Thus, exposure to residues may be reduced by discarding any juices which come from the edible tissues as they are cooked. Between the various agents affecting antibiotics residue after the cooking process, it was found that cooking time and temperature can play major roles about antibiotic residue reduction while cooking food. Also, additional separate residue detection experiments on the metabolites of these drugs must be done, that can be produced after cooking and toxicology experiments must be performed for detection of their effects on human bodies.

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