

Effect of roasting, boiling and microwaving cooking method on Doxycycline residues in edible tissues of poultry by microbial method

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Abstract— The purpose of this study was to determine the effects of different cooking processes like boiling, roasting and microwaving on doxycycline residues in chicken muscle, liver and gizzard tissues of broiler chickens. Each of chicks was fed by water and food with %0.1 of doxycycline in their drinking water for consecutive 5 days. Then; three locations were sampled aseptically from each carcass: breast muscle; liver and gizzard. Doxycycline residue was analysed using microbial inhibition method by plates seeded with *Escherichia coli*. After doing different phases of the test on raw samples, the positive raw samples cooked by various cooking procedures and we surveyed cooked samples with similar method again for the detection of residue. The results showed a reduction in concentration of doxycycline residue after different cooking processes and a part of residue in boiling process excreted from tissue to cooking fluid. Between the various agents affecting antibiotics residue after cooking process, cooking time and temperature can play major role about antibiotic residue reduction while food cooking. Regarding to the results of this study, we can concluded that cooking processes don't guarantee a full elimination of these drug present in condemned animals and it can only decrease it's amounts.

Keywords—cooking, Doxycycline, residue

INTRODUCTION

Tetracycline antibiotics (TCAs) have a broad range of activity against variety of Gram -positive and Gram-negative bacteria and have low cost. For these reasons, TCAs are widely used in veterinary medicine for preventing and treating several diseases and for promoting growth in cattle and poultry (Bogialli and Di Corcia, 2009). Furthermore, they are easy to administer and are effective through oral dosing via water and feed. Tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DOX) are four members of this antibiotic group. In

recent years, the abundant and in some cases improper use of TCs has resulted in the presence of residues in edible animal tissues, which has harmful effects on consumer's health, such as allergic reactions, liver damage, yellowing of teeth, and gastrointestinal disturbance (Debuf, 1998; Joint FAO/WHO Expert Committee on Food Additives, 2002; Jing et al., 2009).

Doxycycline (DOX) is a broad-spectrum antibiotic from tetracycline group that is widely used in the treatment of respiratory tract infections and infectious diseases caused by *Rickettsiae*, *Mycoplasmas* and *Chlamydia* in various species (Hardman et al., 2001; Kirbiš, 2006).

The detection of antibacterial residues in food requires screening methods sensitive at antibiotic concentrations close to the Maximum Residue Limit (MRL). The European Union (EU) legislation on veterinary drug residues established, in 1997, provisional maximum residue limits (MRL) for DOX in bovine, porcine and poultry at 600 µg kg⁻¹ in kidney, 300 µg kg⁻¹ in liver, skin and fat and 100 µg kg⁻¹ in muscle (Croubels et al., 1998; Fuselier et al., 1998).

An efficient screening method for the detection of antibacterial residues in food needs to be low-cost and high-throughput, able to effectively identify potential noncompliant samples from a large set of negative samples. Microbial inhibitions assays were the earliest methods used for the detection of antibiotic residues and they are still widely used. They are very cost-effective and in contrast to, for example, immunological or receptor-based tests, and allow detection of a wide spectrum of antibiotics (Aerts, M.M.L., et al., 1995; Haasnoot, W. et al., 1999; Pikkemaat, M.G., 2009). 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. A positive result should be confirmed with chemical or physical methods (Ferrini, A.M et al. 2006; Kirbiš, A., 2006). A plate test consists of a layer of inoculated nutrient agar, with samples applied on top of the layer, or in wells in the agar. Bacterial growth will turn the agar into an opaque layer, which yields a clear growth-inhibited area around the sample if it contains antimicrobial substances. In Europe this has been the main test format since screening of slaughter animals for the presence of antibiotics started (Pikkemaat, M. G., 2009). On the basis of other researches, the plate seeded with *Bacillus subtilis* is suitable for detection of tetracycline residues (Kirbiš A. 2006; Chang, C.S. et al. 2000). Between 1995 and 1999, Rose M.D. 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 foods-producing animals are always cooked before consumption and the variations in doxycycline levels in the tissue are dependent on type of cooking, more findings about the effect of cooking on doxycycline residue are needed to accurately determine consumer exposure to this drug.

Material & methods

Chickens and drug administration:

Sixty broiler chickens (aged 20 days) were randomly divided into 2 groups; control group and case group; each containing 30 chicks. In order to remove any probable antibiotic residues from chicken's body; they were fed by feed and water free of antibiotics for around 10 days. Each of chicks in case groups were fed by water and feed with %0.1 of doxycycline in their drinking water for consecutive 5 days and chicks in control group were fed by similar water and feed but without doxycycline for similar period.

Preparation of samples:

After the 5th day of the drug administration, chickens were slaughtered and breast muscles; livers and gizzards were sampled aseptically from each carcass. After notation of samples characteristics; they were placed 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 well done in an electric Oven (Memmert, Germany) at 200°C for the specified time 25, 40 and 60 min for liver, muscle and gizzard samples respectively, removed, and allowed to cool. No juices, which drains from the samples as they were cooked, were collected. The cooked muscle had a "well done" appearance.

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:

Test organism that used in this study were *Bacillus subtilis* (PTCC 1365) and the used agar Medium was Muller Hinton agar (Quelab, England) and the pH of the this medium were adjusted to pH=6 with sodium hydroxide and acid acidic and 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 *B. subtilis* in plates. Raw samples disks (diameter 2 mm) were put on each plates also we put a paper disk as negative control. A positive raw sample is indicated by a complete inhibition of growth in an annular zone not less than 2mm wide around the disc. Less than 2mm of inhibitory zone indicated negative result (Myllyniemi, 2001). Results of inhibition zones diameter was read by digital caliper.

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 boiling process of samples. After all samples were put onto the plates, plates were incubated at 37°C for 24 hrs.

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 showed in below table. We see that all cooking processes can lead to a reduction ($p < 0.01$) in diameter of inhibition zones in cooked samples rather than raw samples.

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

	muscle	liver	gizzard
Raw	12.3±0.47d	11.3±1.49c	8.4±2.24b
Boiled	8 ± 0.42 c	0.0 ±0.0a	0.0 ±0.0a
Boiling fluid	6.6 ±1.37c	0.0 ±0.0a	0.8 ± 0.8 a
Micro waved	0.0 ±0.0 a	0.0 ±0.0 a	3.14 ±1.03 a
Roasted	3.1 ± 0.69b	3.9 ±0.46 b	1.3 ± 0.87a

a, b, c and d : Differences between the means that have common letters are significant($p<0.01$).

Discussion

The microbiological screening tests are essentially qualitative screening test, which detects any tissues substance with the property of bacterial inhibition. The advantages of these tests are quite simplicity, inexpensiveness, sensitivity, reliability, and they don't need for high skill of operator. They also have the advantage of being multi-residue tests because inhibition is caused by a wide range of antibiotics. In the microbial test, observation of inhibition zones is possible when antibiotics residue is above MRL because this test can't detect amounts of residues below or around allowable amounts.

According to the results of our study, maximum mean inhibitory zone in cooked muscle samples related to boiling process and minimum mean inhibitory zone regarded to microwaving method. Thus, the most reduction of doxycycline residues in cooked muscle samples related to microwaving process and we found the highest detectable amount of the residue in boiling process and boiling fluid of cooked muscle. A part of residue excreted from tissue to cooking fluid in boiling process about muscle and gizzard samples.

The roasting process has the least effect on the reduction of doxycycline residue in liver samples and we didn't see any Inhibitory zones around cooked liver samples about other cooking methods.

Boiling process about gizzard tissue was led to the minimum mean inhibitory zone around cooked gizzard samples and maximum mean inhibitory zone related to

microwaving process. Thus, the most reduction of doxycycline residues in cooked gizzard samples related to boiling process (table).

The difference between mean inhibitory zone of raw and cooked samples in various cooking processes were not significant ($p<0.01$); also, the differences between various cooking methods were not significant about muscle tissue while it was significant about gizzard samples ($p<0.01$). The difference between boiling process and microwaving method was significant about liver samples ($p<0.01$) (table).

The results of our study are consistent with other studies on the fate of doxycycline residue. Based on a research about the effect of cooking processes on tetracycline compounds in poultry products in the eastern province of Saudi Arabia, the authors mentioned that the MRL for doxycycline was exceeded in raw liver samples in 3 poultry farms. However, after cooking (100°C for 20min) the mean detectable concentrations of this drug were decreased to below MRL. Mean concentrations of doxycycline were also above the MRL in raw muscle obtained from 5 farms. However, after cooking (100°C for 20min), the MRL of these drug was exceeded only in 2 of the farms (Al Ghamdi, et al. 2000).

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 proved that doxycycline was degraded (less than 10% remaining activity) after the sterilization step (Van egmond et al., 2000) or in a study on Thermostability of doxycycline at ultrahigh temperatures Whereas low-temperature-long-time treatments (conventional sterilization) would destroy >98% of the initial concentration of the residues of the doxycycline, high-temperature-short-time treatments (UHT) would leave unaltered doxycycline in the 50-90% range (Hassani, et al ,2008). The results of these researches confirm the findings of our study about the decrease of doxycyclin activity after cooking procedure.

CONCLUSIONS

We can concluded that cooking processes don't guarantee a full break-down of these drug present in condemned animals and it can only decrease it's amounts and a part of residue in boiling process excreted from tissue to cooking fluid in 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 cooking process, cooking time and temperature can play major role about antibiotic residue reduction while food cooking .

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