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Full Length Research Paper

Effect of roasting, boiling and microwaving cooking method on sulfadiazine + trimethoprim residues in edible tissues of broiler by microbial inhibition method

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The purpose of this study was to determine the effects of different cooking processes like boiling, roasting and microwaving on sulfadiazine + trimethoprim residues in edible tissues of broiler chickens. Each of chicks was fed by water and food with 0.05% of sulfadiazine + trimethoprim in their drinking water for consecutive 5 days. After the 5th day of the drug administration, they were killed and breast muscle; liver and gizzard were sampled aseptically from each carcass. The residue was analyzed using microbial inhibition test by plates seeded with *Bacillus subtilis*. The positive raw samples cooked by various cooking procedures (roasting, boiling and microwaving) . Then, we surveyed cooked samples for the present of residue. The results show the reduction in concentration of sulfadiazine + trimethoprim residue after different cooking processes. The most reduction of sulfadiazine + trimethoprim residues in cooked muscle samples related to microwaving process and a part of residue excreted from tissue to cooking fluid in boiling process. Muscle samples had the most resistance to residue reduction rather than other tissues in boiling and roasting processes. The reduction effect of all cooking processes on liver and gizzard samples was greater than muscle samples and the inhibitory zone around all cooked liver and gizzard samples were not detectable. Regarding to the results of this study, we can concluded that cooking processes do not guarantee a full break-down of these drugs present in condemned animals. Between the various agents affecting antibiotics residue after cooking process, cooking time and temperature can play major role about antibiotic residue reduction.

Key words: Cooking, sulfadiazine, trimethoprim, residue, edible tissue, broiler.

INTRODUCTION

The sulfonamide family includes a broad spectrum of synthetic antibiotics used in poultry farming as growth promotion, prophylaxis and treatment of bacterial and protozoal diseases (White et al., 1981; Morita and Akasi, 1991). Trimethoprim (TMP) is a dihydrofolate-reductase inhibitor. In veterinary medicines, TMP is commonly administered in combination with a sulfonamide and exert a bactericidal activity against many Gram-positive and Gram-negative bacteria (Silva, 2002; Bushby, 1980). slaughtering; they generate serious problems for human health, such as allergic or toxic reactions (Pastor-Navarro

Most of the sulfonamides have a relatively long half-life, if the proper withdrawal periods are not observed before et al., 2009; Zhang et al., 2008) . Their residues in food are of great concern because of their potential carcinogenic character and their contributing to the development of antibiotic resistance in humans. Monitoring of such residues in products for human consumption and in slaughtered animals has become one of the most important duties for public health agencies (Samanidou et al., 2008).

The detection of antibacterial residues in food requires screening methods sensitive at antibiotic concentrations close to the maximum residue limit (MRL) (Fuselier et al., 1999). In European Union, Canada and the USA, the MRL of total sulfonamides in edible tissues is 100 g / kg^{-1}, and 20 g / kg^{-1} in Japan (Zhang et al., 2008); Also,

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EU has established the MRL for trimethoprim about 50 g / kg^{-1} in edible tissues (Pikkemaat, 2009).

An efficient screening method needs to be low-cost and high-throughput. The vast majority of the screening methods used for monitoring the presence of antimicrobial compounds today are still microbial inhibition tests. Microbial inhibitions assays were the earliest methods used for the detection of antibiotic residues and they are very cost-effective in contrast to, for example, immunological or receptor-based tests, and still widely used for detection of a wide spectrum of antibiotics (Aerts et al., 1995; Haasnoot et al., 1999; Pikkemaat, 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 et al., 2006; Kirbiš, 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, 2009).

On the basis of other researches, the plate seeded with *Bacillus subtilis* is suitable for detection of sulfonamides residues (Tsai and Kondo, 2001; Chang et al., 2000). Since the most of foods-producing animals are always cooked before consumption and the variations in sulfonamides levels in the tissue are dependant on type of cooking (Furusawa and Hanabusa, 2006); more findings about the effect of cooking on sulfadiazine + trimethoprim residue are needed to accurately determine consumer exposure to this drug. So, the aim of this study was to determine the effects of different cooking processes on sulfadiazine + trimethoprim residues in chicken muscle, liver and gizzard tissues of broiler chickens.

MATERIALS AND METHODS

Chickens and drug administration

Sixty male broiler chickens (Ross 308) (3 weeks old) were randomly divided into 2 groups; each containing 30 chicks. In orders to remove their bodies from the probable antibiotic residue; they were fed by free antibiotic food for around 10 days. Each of chicks in case groups were fed by water and food with 0.025 % of sulfadiazine + trimethoprim in their drinking water for consecutive 5 days and chicks in control group were fed by similar water and food but without sulfadiazine + trimethoprim for 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 samples 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 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

Test organism that used in this study were *B. subtilis* (PTCC1365) and the used agar Medium was Muller Hinton agar (Quelab, England) and this medium were adjusted to pH = 7.2 with sodium hydroxide and acid acidic and autoclaved as indicated by the manufacturers. Sterile Petri dishes (diameter 90 mm) were filled with 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 for negative control. 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, 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 h.

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

After doing different phases of the test, comparison of the effects of different cooking methods on the mean diameter of inhibition zones (mean ± SE) around raw and

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

	Muscle	Liver	Gizzard
Raw	7.0 ± 1.94 b	1.8 ± 0.92 a	1.7 ± 0.87 a
Boiled	2.3 ± 1.52 ab	0.0 ± 0.0 b	0.0 ± 0.0 b
Boiling fluid	2.4 ±1.52 ab	0.0 ±0.0 b	0.0 ± 0.0 b
Microwaved	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Roasted	1.8 ± 0.95 ab	0.0 ± 0.0 b	0.0 ± 0.0 b

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

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

	Boiled	Boiling fluid	Micro waved	Roasted
Muscle	2.3 ±1.52 [a]	2.4 ± 1.52[a]	0.0 ± 0.0 [a]	1.8 ± 0.95[a]
Liver	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]
Gizzard	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]

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

cooked samples are showed in Table 1. We see 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.

DISCUSSION

The microbiological screening tests are essentially qualitative 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 do not 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.

According to the results of our study, maximum mean inhibitory zone in cooked muscle samples related to boiling fluid and minimum mean inhibitory zone regarded to microwaving method. Thus, the most reduction of sulfadiazine + trimethoprim 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 and a part of residue in boiling process excreted from tissue to cooking fluid in boiling process. But, the reduction effect of all cooking processes on liver and gizzard samples was greater than muscle samples and the inhibitory zone around all cooked liver and gizzard samples were not detectable and the amount of residue decreased below the MRL. Also, the difference between mean inhibitory zone about all cooked samples in various cooking processes were significant ($p < 0.01$) (Table 1). Muscle samples had the most resistance to residue reduction rather than other tissues in boiling and roasting processes. Also, the difference between mean inhibitory zone of various cooked tissues were significant ($p < 0.01$) (Table 2).

Based on a research about cooking effects on sulfonamides residues in chicken thigh muscle, the scientists mentioned that by boiling procedure, the reductions of four the sulfonamides (sulfadiazine, sulfamethoxazole, sulfamonomethoxine and sulfaquinoxaline) were 45 to 61% in 12 min. The reductions of four sulfonamides in muscles cooked by microwaving (500 W for 1 min) were rapid, and their residues were reduced 35 to 41% in 1 min and the reducing effect on sulfonamides residues in the muscles by microwave cooking was greater than those by other cooking methods (Furusawa et al., 2001). The results of boiling and microwaving in this research confirm the findings of our study about the reduction effect of these processes and the microwaving process had the greatest effect on sulfadiazine + trimethoprim reside in our muscle samples.

In a study about the effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish, It has been demonstrated that cooking caused an average 54.0% reduction of ormetoprim and 46.1% reduction of sulfadimethoxine from raw fillet of fish (Xu et al., 2002).

There are other reports about the reduction effect of cooking processes about the family of sulfonamides. The fish meat with sulfamethazin residue microwaved under 2650 MHz had a residual level of 10.0~12.5% after 5 min treatment. This meant almost 90% losses in this case. After roasting at 200°C for 20 min, it had a residual level of sulfamethazin about 27.0~38.5%, and about 15~23.5% by roast treatment at 200°C for 30 min (Lan et al., 2001).

In another study, the mean remaining activity after the sterilization step (134°C) was for sulfamethazine 38% and for sulfamethoxazole less than 10% remaining activity (Van Egmond et al., 2000).

It has been demonstrated that the most significant reasons for reduction of residual level and increase of loss are temperature and time effects (Lan et al., 2001). Judging from the reduction of residual levels which are caused by the various heat treatments, it has attributed to the degradation possibility of sulfonamides and the binding of them with proteins of tissues and this hypothesis was proposed by other scientists about sulphamethazine residue (Lan et al., 2001; Papapanagiotou et al., 2005).

According to the results of this paper and findings of another researches about effects of different cooking procedures on antibiotic residue in food stuff, we can concluded that cooking processes do not guarantee a full break-down of residues of veterinary drugs present in condemned animals and they can only decrease their amounts and the most 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. Also, additional separately residue detection experiments on the metabolites of these drugs must be done that can be produced after cooking and toxicology experiments must performed for detection of their effects them on human bodies.

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