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Effect of Roasting Process on Antibiotic Residues in Edible Tissues of Poultry by FPT Method

[1]A. Javadi, [1]H. Mirzaei and [2]S.A. Khatibi
[1]Department of Food Hygiene, Faculty of Veterinary Science,
[2]Young Researchers Club, Islamic Azad University, Tabriz Branch, Iran

Abstract: Regarding to vast application of the antibiotic drugs in animals, quality control of food stuff, from view of antibiotic residues is necessary. Existence of antibiotic residues in food stuff, especially meat and their transforming to the body of consumers are the cause of some effects such as bacterial resistance, allergic reactions, toxicity, carcinogenic effects and disturbing of natural micro flora of intestine. This study presents, the net change by roasting process on antibiotics residues in edible tissues of poultry. Four-plate test is one of the microbiological methods for detection of antibiotic residues in food stuff, which is at the base of inhibition zone formation around the samples disks in four culture media with different pH and test bacteria. For this purpose, 40 carcasses were collected on each of eight visits from Tabriz poultry slaughter houses. Four locations were sampled aseptically from each carcass: breast muscle; skin; liver; gizzard. After doing different phases of four-plate test on raw samples, the positive raw samples incubated in 200°C. Incubation time regarding to usual a complete cooking time were as follows: muscle 40 min; skin 15 min; liver 25 min; gizzard 60 min then, we surveyed samples by FPT method again for present of antibiotic residue. None of the positive raw samples had antibiotic residue more than maximum residue limit after roasting ($p<0.05$) and we didn't see any inhibition zones around roasted samples on agar medium. Regarding to the results of this study, we can conclude that enough roasting temperature and time can have a great effect on antibiotic residues losses and provides an additional margin of safety for consumers.

Key words: Roasting, antibiotic, residue, poultry, edible, tissue, FPT

INTRODUCTION

As with human medicine, veterinary drugs are widely used in animal industries, since 1950s. Antibiotics are normally administered by veterinarians for treatment, prevention and growth promotion in poultry (Dipeolu *et al*., 2002; Botsoglou and Fletouris, 2001). Existence of antibiotic residues in food stuff, especially meat can create some side effects in the body of human. Among them are sensitivity to antibiotics, allergic reactions and imbalance of intestinal microflora, bacterial resistance to antibiotics in microorganisms and losses in the food industry (Cunha, 2001; Kirbis, 2006).

Also, antibiotics consumption in third world countries and especially in Iran is irregular and it doesn't remark to withdrawal times of antibiotics. So, quality control of food stuff regarding to antibiotic residues, is necessary. 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 can not be used to identify individual antibiotics a positive result should be confirmed with chemical or physical methods (Ferrini *et al*., 2006; Kirbis, 2006). Microbial methods are relatively inexpensive, easy to use, do not require expensive equipment and can be efficiently adopted by laboratory staff. Although, minimal expenditure is a significant factor of analyses, no test is valuable if it does not give reliable

Corresponding Author: A. Javadi, Department of Food Hygiene, Faculty of Veterinary Science, Islamic Azad University, Tabriz Branch, Iran

results (Okerman *et al*., 1998, 2004). Four plate test is a reference microbiological method for the screening of antibiotic residues and it still used in European countries (Heitzman, 1994). It can detect the presence of a number of antibiotic residues at the same time, including β-lactam, Tetracycline, Macrolides, Aminoglycosides and Sulfonamides (Koenen-Dierick *et al*., 1995; Nolan *et al*., 2000; Chang *et al*., 2000). Between 1995 and 1999, Rose *et al*. (1999) 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, more findings about the effect of cooking on antibiotic residue are needed to accurately determine consumer exposure to these drugs.

MATERIALS AND METHODS

For this study, 40 carcasses randomly were collected on each of eight visits from Tabriz poultry slaughter houses in Iran in 2007. Four locations were sampled aseptically from each carcass: breast muscle; skin; liver; gizzard and average weights of raw samples were 100 g. After notation of samples characteristics; we placed them in sterile polyethylene containers. The used detection method was four plate test (Heitzman, 1994) in the study, an agar diffusion test in which an organism sensitive to the antibacterial substances was inoculated into an agar medium in a Petridis. Test organisms that used in this study were *Bacillus subtilis* (PTCC1365) and *Micrococcus lutes* (PTCC 1169) and the used agar Medium was Muller Hinton agar (Quelab, England) and this medium were adjusted to pH 6,7.2 and 8 with sodium hydroxide and acid acidic and autoclaved as indicated by the manufacturers. Sterile Petri dishes (diameter 90 mm) were filled with 5 mL of the prepared culture medium then we seeded *B. subtilis* in test agar pH 6, pH = 7.2 and pH = 8 and *M. luteuse* was seeded in test agar pH = 8. Raw samples disks (diameter 2 mm) were put on each plates also we put a paper disk for negative control. After the samples were put onto the plates, plates with *B. subtilis* were incubated at 30°C for 18 h and plates with *M. luteus* incubated at 37°C for 24 h. Plates containing *B. subtilis* pH = 6 are to detect in particular beta-lactam and tetracycline residues. Plates containing *B. subtilis* pH = 7.2 and *B. subtilis* pH = 8 are to detect particular sulfonamides and Amninoglycosides residues, respectively. Plates containing *M. luteus* pH = 8 is to detect particular Beta-lactam and Macrolides residues (Chang *et al*., 2000).

A positive raw sample is indicated by a complete inhibition of growth in an annular zone not <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.

The positive raw samples was placed in aluminum foil on a metal tray and incubated in 200°C in the center of electric oven (Memmert, Germany). Regarding to usual and complete cooking time, roasting time was as follows: muscle 40 min; skin 15 min; liver 25 min; gizzard 60 min then, we perused roasted samples for present of antibiotic residue by FPT method like raw samples.

RESULTS

After doing different phases of four-plate test, from total 40 raw muscle samples, 25 cases (62/5%) (Table 1); from total 40 raw liver samples, 40 cases (100%) (Table 2); from total 40 raw skin samples, 21 cases (52/5%) (Table 3) and from 40 raw gizzard samples, 15 cases (37/5%) antibiotic residues were diagnosed to be above MRL (Table 4), but none of the positive raw samples from muscle , liver, skin and gizzard had antibiotic residue more than maximum residue limit after roasting (Table 1-4). Comparison between mean diameter of inhibition zones around samples had a significant different before and after roasting ($p<0.05$). These results analyzed analyzed by t-test and SPSS software (version 15) and it's illustrated in Table 1-4.

Table 1: Comparison of mean inhibition zones between raw and roasted skin samples by t-test

Samples	Groups	N	Mean[a]	SD	SEM	df	t-test	Sig. (2-tailed)[b]
Bacillus subtilis	Raw	40	1.25	3.07	0.048	39	2.58	0.001
pH = 6	Roasted[c]	21	0	0	0			
Bacillus subtilis	Raw	40	2.22	3.48	0.55	39	4.04	0.000
pH = 7/2	Roasted	21	0	0	0			
Bacillus subtilis	Raw	40	2.78	3.66	0.57	39	4.81	0.000
pH = 8	Roasted	21	0	0	0			
Micrococcus luteus	Raw	40	1.94	3.44	0.54	39	3.56	0.014
pH = 8	Roasted	21	0	0	0			

[a]Mean diameter of inhibition zones around raw and roasted samples to millimeter; [b]Differences in the inhibition zones diameter between raw and roasted samples are significant ($p<0.05$); [c]For 15 min

Table 2: Comparison of mean inhibition zones between raw and roasted muscle samples by t-test

Samples	Groups	N	Mean[a]	SD	SEM	df	t-test	Sig. (2-tailed)[b]
Bacillus subtilis	Raw	40	2.27	3.57	0.56	39	4.02	0.000
pH = 6	Roasted[c]	25	0	0	0			
Bacillus subtilis	Raw	40	2.00	3.53	0.65	39	3.58	0.001
pH = 7/2	Roasted	25	0	0	0			
Bacillus subtilis	Raw	40	4.68	4.1	0.55	39	7.17	0.000
pH = 8	Roasted	25	0	0	0			
Micrococcus luteus	Raw	40	1.22	2.97	0.56	39	2.60	0.013
pH = 8	Roasted	25	0	0	0			

[a]Mean diameter of inhibition zones around raw and roasted samples to millimeter; [b]Differences in the inhibition zones diameter between raw and roasted samples are significant ($p<0.05$); [c]For 40 min

Table 3: Comparison of mean inhibition zones between raw and roasted gizzard samples by t-test

Samples	Groups	N	Mean[a]	SD	SEM	df	t-test	Sig. (2-tailed)
Bacillus subtilis	Raw	40	1.27	3.44	0.54	39	2.34	0.024
pH = 6	Roasted[c]	15	0	0	0			
Bacillus subtilis	Raw	40	1.19	2.91	0.46	39	2.59	0.013
pH = 7/2	Roasted	15	0	0	0			
Bacillus subtilis	Raw	40	1.97	3.50	0.055	39	3.56	0.001
pH = 8	Roasted	15	0	0	0			
Micrococcus luteus	Raw	40	1.99	3.76	0.059	39	3.34	0.002
pH = 8	Roasted	15	0	0	0			

[a]Mean diameter of inhibition zones around raw and roasted samples to millimeter, [b]Differences in the inhibition zones diameter between raw and roasted samples are significant ($p<0.05$). [c]For 60 min

Table 4: Comparison of mean inhibition zones between raw and roasted liver samples by t-test

Samples	Groups	N	Mean[a]	SD	SEM	df	t-test	Sig. (2-tailed)
Bacillus subtilis	Raw	40	3.94	4.34	0.23	39	6.39	0.000
pH = 6	Roasted[c]	40	0	0	0			
Bacillus subtilis	Raw	40	3.32	3.94	0.61	39	5.32	0.000
pH = 7/2	Roasted	40	0	0	0			
Bacillus subtilis	Raw	40	5.44	3.91	0.62	39	8.78	0.000
pH = 8	Roasted	40	0	0	0			
Micrococcus luteus	Raw	40	9.02	1.45	0.029	39	9.18	0.000
pH = 8	Roasted	40	0	0	0			

[a]Mean diameter of inhibition zones around raw and roasted samples to millimeter, [b]Differences in the inhibition zones diameter between raw and roasted samples are significant ($p<0.05$). [c]For 25 min

DISCUSSION

The European Four Plate Test (FPT) is used to establish whether and where antimicrobial residues accumulate in the tissues of commercial animal farming. It is essentially a qualitative screening test, which detects any tissues substance with the property of bacterial inhibition. This test can detect five groups of antibiotics like Betalactams, Tetracyclines, Sulfonamides, Aminoglycosids and Macrolydes (Chang *et al*., 2000). On the basis of international standards and obtained findings from *B. subtilis* and *M. luteus* sensitivity test in comparison with maximum residue limit in carcasses, we found that observation of inhibition zones is possible when antibiotics residue is above MRL because this test can't detect amounts of residues below or around permissible amounts (Myllyniemi *et al*., 2001; Chang *et al*., 2000). Therefore, we can say that antibiotics residue in roasted samples on plate agar, reached to below or around allowable limit after roasting in 200°C.

In this survey, penicillin's group activity in test agar pH = 6 with *B. subtilis* and test agar pH = 8 with *M. luteuse* decreased and we didn't observe any inhibition zones around roasted samples in these plates (Table 1-4). It has proved that cooking of hamburger, steaks and port chops can result to rare and medium change in benzyl penicillin's activity but only a small percent of original activity remained after well done cooking (Rose *et al*., 1997).

Based on a study on the residues of tetracycline compounds in Chicken muscle and liver samples were collected from 33 broiler and 5 layers farms in the eastern province of Saudi Arabia over a period of 2 years. The MRL for tetracycline, chlortetracycline and doxycyciline was also exceeded in raw liver samples in 21 farms. However, after cooking in boiling water for 20 min, the mean detectable concentrations of these three drugs were decreased to below MRL except for CHT in 5 farms. Furthermore, mean concentrations of TET, CHT and DXC were also, above the MRL in raw muscle obtained from

10, 9 and 5 farms, respectively. However, after cooking, the MRL of these drugs was exceeded only in 3, 6 and 2 of the farms, respectively (Al-Ghamdi *et al*., 2000).

In the present study, we couldn't detect tetracycline residues in test agar pH = 6 with *B. subtilis* after roasting in 200°C (Table 1-4). In comparison with study of MS Al-Ghamdi *et al*. (2000), the raw liver and muscles cooked in higher temperature and longer time.

In a study about neomycin residues in eggs and stability of residues after cooking, it has proved that Frying eggs caused little or no loss of activity, poaching resulted in 25% loss and soft boiling and hard boiling caused little or no loss of applied activity (Katz and Levine, 1978).

But, in the present study we couldn't determined any inhibition zone due to aminoglycozid group in test agar pH = 8 with *B. subtiltis* around roasted samples (Table 1-4).

Residues of fluorocinolones, enrofloxacin and ciprofloxacine are detectable only with additional plate with *Escherichia coli*, so, we couldn't detect residues of these antibiotics groups in raw and roasted samples in this study.

It is demonstrated that, enrofloxacin remained stable for 3 h in water when heated at 100°C and cooking procedures did not affect enrofloxacin residues, which remained stable during heating (Lolo *et al*., 2006). Also, it has proved that norfloxacin residues are detectable by high performance liquid chromatography in 35.0 and 56.7% of raw antibiotic-residue-positive muscles and livers, respectively (Zaki *et al*., 2000).

According to a research about cooking effects on sulfonamide residues in chicken thigh muscle, these Sulfonamides (SAs) were fed to chickens at a dietary concentration of 100 mg kg^{-1} (each drug) for 7 successive days. The residues of sulfamethoxazole, sulfamonom-ethoxin and sulfaquinoxaline exept sulfadiazine in the muscle cooked by roasting at 170°C were reduced to 38-54% in 12 min. However, the reduction of SDZ residue was only 4% in 12 min. Also, residue of these antibiotics in both raw and cooked samples was higher than maximum residue limit (Furusawa *et al*., 2002).

In the present survey, we didn't observe any inhibition zones in test agar pH = 7.2 with *B. subtilis* that could show sulfonamides residue in samples (Table 1-4). Reason of difference between our results with the findings of said study may correspond to difference of cooking degree and time.

Another reason of different can correspond to feeding period of this drugs in poultry and because of occurrence of some problems like vitamin K deficiency with feeing for >2 or 3 days and another reason can correspond to detection method of residues that detection technique was HPLC method in study of N. Furusawa *et al*. (2002) that this said method is more quantative and precise than FPT and it even can determine amounts of residues below MRL.

According to results of this study and findings of another researches about effects of different cooking processes on antibiotic residue in food stuff, we can concluded that cooking process can't annihilate total amounts of these drugs and it can only decrease their amounts. Between various agents affecting antibiotics residue after cooking process, cooking time and temperature can play major role about antibiotic residue decreasing, while food cooking. Therefore, use of cooking processes that have higher temperature and longer time can lead to the most decrease in antibiotics residue in food stuff and it can provide an additional margin of safety for consumers but the effects of metabolites of antibiotics residue that can be produced after cooking must be studied in human bodies by toxicology experiments in next researches.

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