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BACTERIOLOGICAL ASSESSMENT OF FRESHLY SLAUGHTERED CHICKEN AND A TRIAL FOR IMPROVEMENT (With 2 Tables and 3 Figures)

By

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التقييم البكتريولوجي للدجاج المذبوح الطازج ومحاولة تحسينه

تشرأح خليل إبراهيم ميره ، عادل اسكندر عبد الرحمن

استخدم عدد خمسون عينة بواقع عشرة من كل من الدواجن الكاملة حديثة الذبح والصدور والأوراك والفيلية والكبد والقوانص. وتم عمل العد الكلي للميكروبات الهوائية والعد الكلي للميكروبات القولونية والمعوية والميكروب المكور العنقودي الذهبي وعزل وتصنيف ميكروب السالمونيلا. أظهرت النتائج أن عدد هذه الميكروبات سجل أعلى معدلات في الكبد والقوانص تلاها عينات الدواجن الكاملة. كما تم عزل عترات مختلفة من ميكروب السالمونيلا من جميع عينات الدواجن ومنتجاتها بنسب مختلفة. ولتقييم كفاءة استخدام مياه الأوزون على الحالة البكتيرية للدواجن المذبوحة أظهرت النتائج أن غمس الدواجن المذبوحة في الأوزون المائي لمدة ٣٠ دقيقة أدى إلى إنقاص العد الكلي للميكروبات الهوائية والميكروب القولوني بمعدل أكبر من ٣ لوغار يتم خلية بكتيرية/جم من العينة والميكروب المكور العنقودي الذهبي بمعدل ٢,٥ لوغار يتم خلية بكتيرية/جم من العينة. وأيضا قلل عد ميكروب السالمونيلا ٣,٩ لوغار يتم خلية بكتيرية/جم من العينة للعينات التي عمرت لمدة ٢٠ دقيقة وقلها أكثر من ٥ لوغار يتم خلية بكتيرية/جم من العينة بعد الغمر لمدة ٣٠ دقيقة.

SUMMARY

Fifty samples of fresh whole chicken carcasses, breast, thigh, fillets and giblets were examined bacteriologically for Aerobic Plate Count (APC), coliforms count (MPN), Enterobacteriaceae count, *Staphylococcus aureus* count and isolation of *Salmonella*. Results indicated that chicken and its products, giblet samples exhibited the highest bacterial population followed by the whole carcasses samples. *Salmonellae* were isolated from all samples by different percentages. To evaluate the efficiency of ozonated water on bacterial status of chicken carcasses, the results indicated that immersion of chicken carcasses in ozonated water

for 30 minutes was more effective in reducing the bacterial population of APC and coliforms count by more than 3 log cfu/g respectively and *Staphylococcus aureus* was reduced by 2.5 log cfu/g. *Salmonella typhimurium* population was reduced by 3.9 log cfu/gm after treatment with ozonated water for 20 minutes and by nearly 5 log cfu/g for 30 minutes treatment.

Key words: *Chicken carcasses, giblets, APC, coliform, Enterobacteriaceae, S.aureus, salmonellae*

INTRODUCTION

In poultry processing, live birds enter the abattoir carrying large numbers of microorganisms on their feather, feet and skin, with the feather being the most contaminated (Kotula and Pandya, 1995).

Although the feathers are removed during the defeathering stage, the preceding of scalding process and the defeathering process itself allow cross-contamination of carcasses skin with bacteria from feather, feet and guts, as well as equipments and environmental sources, such as water and air (Mead, 1989; Kotula and Pandya, 1995).

The food safety and inspection service (FSIS) conducted a survey (Green, 1987) of poultry processing plants which showed that 3 to 4% of broilers coming into processing plant are *Salmonellae* positive, whereas about 35% of processed birds leaving the plant are positive for this human enteric pathogen. These statistics indicate that cross-contamination occur during processing between normal and diseased broilers.

In Egypt, poultry meat industry show a competitive advantage over other meat industries because of rapid rate of improvement, intensive use costs, highly automatic processing plants, competition and economic scale.

The majority of Egyptian prefers to use fresh chickens, chicken parts or giblets, the matter that lead to deal with small scale manual poultry processing shops. These shops didn't implement effective hygienic measures or food safety instruction, since most of the recommended hygienic measures in the processing chain in the modern poultry processing plant are not applicable.

Chlorine is the most commonly used antimicrobial agent in food processing due to its availability, relative low cost and efficiency. One of the major disadvantages of chlorine is its ability to bind to organic material rendering it inefficient in a relatively short period of time,

which requires its constant replenishment (Tsai *et al.*, 1992). Additionally, chlorine is known to produce chloramines which may interfere with the chlorinated compound's activity against bacterial populations (Gelinas and Goulet, 1983).

Ozone act as a powerful oxidizing agent that is 1.5 times stronger than chlorine (Xu, 1999) and is more effective against a variety of microorganisms including vegetative and spore-forming Gram-negative and Gram-positive bacteria, fungi, viruses and protozoa. Also, the U.S Expert committee deemed ozone GRAD and encouraged its use over a broader spectrum of foods in 1997 (Kim and Marshall 1999). More recently, the Food and Drug Administration (FDA) approved the use of ozone as an antimicrobial agent in meat and poultry in aqueous or gaseous form (Federal Register, 2001).

The instability of ozone is considered an advantage as it decomposes rapidly to form oxygen without leaving any residual ozone. As a result, ozone is considered a process rather than a chemical additive (Pryor and Rice, 1999).

Also the use of ozone in the food industry has been investigated for food preservation, shelf life extension, equipments sterilization and improvement of food plant effluents (Kaess and Werdmann, 1968).

Forsythe and Waldroup (1994) reported the economic benefit of ozone in poultry processing plants, such as reduction in water waste purchase, reduction sewage treatment costs and saving in electrical energy from recycling ozonated water. In addition to the economic benefits of water recycling, the use of water with fewer chemical residuals will be favorable to the environment.

Therefore this study focused on the bacterial population, incidence and distribution of some pathogenic organisms on local fresh chicken, chicken parts and giblets obtained from local manual processing poultry shops after slaughter and evaluate the production/processing operation and the using of ozone water as an antimicrobial agent for reducing such bacterial contamination.

MATERIALS and METHODS

PART I: Bacteriological quality of broilers carcasses:

1- Collection of samples:

Fifty samples (10 each) of fresh whole poultry carcasses, breast, thigh, fillets and giblets, were collected from manual processing poultry shops located in Giza Governorate.

2- Preparation of samples:

Bacteria were recovered from carcasses using the whole carcass rinse procedure (Cox *et al.*, 1981).

Samples from the breast, thigh, fillet and giblets (10 g each) were homogenized according to APHA (1992).

3- Bacteriological count and isolation:-

Aerobic Plate Count (APC), coliforms count Most probable Number, Enterobacteriaceae count and *Staphylococcus aureus* count as well as isolation and identification of *Salmonella* were conducted according to the methods approved by APHA (1992). *Salmonella* species were identified in Central Laboratory for Quality Control Poultry Production accredited Laboratory according to ISO 17025 (Ministry of Agriculture-Egypt).

PART II: Decontamination of broilers carcasses by ozone:

Application of ozone:

Ozonated water was obtained from the Egyptian Medical Center (Cairo-Egypt).

A) Effect of ozonated water on bacterial load:

- Two freshly eviscerated broiler carcasses obtained from chicken slaughter shops were sampled using whole carcass rinse technique to determine APC, coliforms, *Staphylococcus aureus* counts as described before.
- Chilled tap water (municipal water source) was preozonated for 25 minutes to obtain 3.0-4.5 ppm aqueous ozone concentration.
- Four broiler carcasses (two per each treatment) were immersed in either ozonated tap water and tap water for 20 and 30 minutes.
- Crushed ice were put around the container of dipping to maintain water temperature between 4-7°C.
- Following chilling, the carcasses were allowed to drain for 15 minutes in the refrigerator at 4-7°C.

B) Effect of ozonated water on *Salmonella typhimurium*:

- *Salmonella typhimurium* strain was propagated in trypticase soya broth at 37°C for 18 hours prior to be inoculated in broiler carcasses. Incubated culture was serially diluted in buffered peptone water to obtain a viable cell population of approximately 7 log cfu/ml.
- Four freshly eviscerated broiler carcasses were obtained from local chicken slaughter shops. The whole carcasses were subjected to Ultra Violet light-treatment for 10 minutes (Cutter and Siragusa, 1994).

- Ultraviolet treated carcasses were spread-inoculated with *Salmonella typhimurium* suspension counting 7 log cfu/ml using sterile cotton swabs.
- contaminated carcasses were allowed to attach for 15 minutes at room temperature in a safety hood to obtain approximately 5 log cfu of pathogen/ml.
- Following the inoculation and attachment, carcasses (two carcasses of each) were submerged in tap water and ozonated water (4-7°C) by using crushed ice around the container.
- Treated carcasses were examined bacteriologically for enumeration of *Salmonella typhimurium* using Xylose Lysine Desoxycholate (XLD, Oxoid CM 469) agar. Three experiment replicates were performed.

RESULTS

Table 1: Mean values ± SE of bacterial count log₁₀ cfu/g* of the examined broiler carcasses (n = 10 of each).

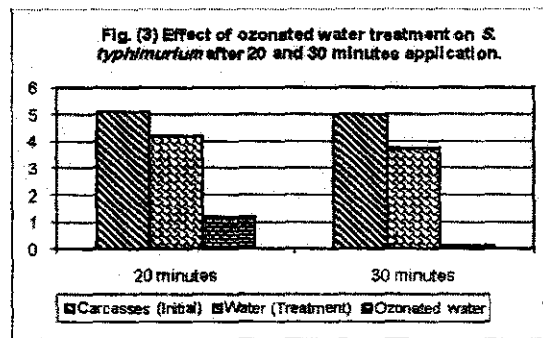
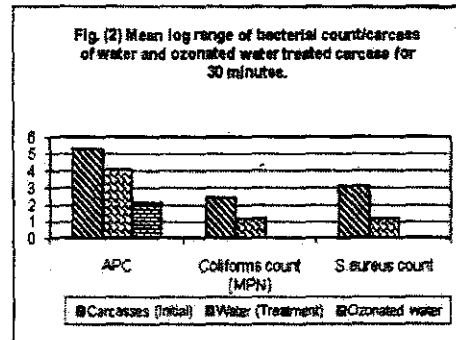
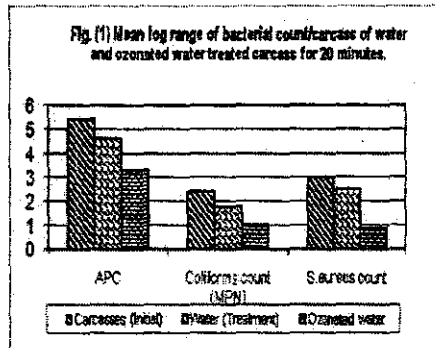
Samples	APC**	Coliforms count (MPN)	Enterobacteriaceae count	<i>Staphylococcus aureus</i> count
Whole carcasses	6.1±0.1	2.7±0.1	4.6±0.1	3.7±0.1
Breast	5.6±0.1	2.2±0.1	4.5±0.1	3.3±0.1
Thigh	5.4±0.2	2.0±0.2	4.2±0.1	3.2±0.1
Fillet	5.6±0.1	2.3±0.1	4.5±0.1	3.4±0.1
Giblets	6.4±0.2	2.9±0.1	4.9±0.2	4.0±0.1

* cfu/g = colony forming unit per gram

** APC = Aerobic Plate Count

Table 2: Incidence of *Salmonellae* species in the examined samples.

Samples (n=10 of each)	Positive samples	Percent (%)	Serotypes
Whole carcasses	4	40	<i>S. typhimurium</i> , <i>S. Kentucky</i> , <i>S. enteritidis</i>
Breast	3	30	<i>S. typhimurium</i> , <i>S. Kentucky</i>
Thigh	1	10	<i>S. enteritidis</i>
Fillet	1	10	<i>S. enteritidis</i>
Giblets	3	30	<i>S. typhimurium</i> , <i>S. infantis</i>



DISCUSSION

Bacteriological quality of the examined samples:

Table (1) shows the bacterial numbers of the whole carcasses, breast, thigh, fillet and giblets samples. Giblets samples consistently exhibited the highest count for all bacterial types (6.4, 2.9, 4.9 and 4.0) for APC, coliforms, Enterbacteriaceae and *Sataphylococcus aureus* followed by the whole carcasses samples. Mean log cfu value of aerobic plate counts (APC) of whole carcasses, breast, thigh and fillet were (6.1, 5.6, 5.4 and 5.6) respectively. These results were nearly similar to that obtained by Lillard (1989) and Capita *et al.* (2001), while higher results were recorded by Lillard *et al.* (1990) and Kolula and Pandya (1995). Moreover, lower results were obtained by Bailey *et al.* (2000) and Patisias *et al.* (2006). The mean log cfu value of coliforms count. (MPN) were (2.7, 2.2, 2.0, and 2.3) for the forementioned samples respectively. These results were coincided with that obtained by Bailey *et al.* (2000) and higher than that obtained by Al-Mohizea *et al.* (1994). While, Russell *et al.* (1995) observed high results.

The mean log of coliforms counts (cfu/g) were high in most of the samples where probably because the skin and the breast of the birds come into contact with the feces contaminated litter on the grow-out facility floors as the birds dust themselves and settle onto their breasts while resting. The source of coliforms bacteria was most likely from fecal contamination occurring during this stage of processing (Notermans *et al.*, 1977).

Enterobacteriaceae mean log cfu/g value were recorded in Table 1 where, whole carcasses, breast, thigh and fillet samples recorded 4.6, 4.5, 4.2 and 4.5 respectively. Similar results were achieved by Lillard (1989) but higher results were recorded by Lillard (1990). In regard to mean log value of *Staphylococcus aureus* count (cfu/g), the forementioned samples recorded 3.7, 3.3, 3.2, and 3.4, respectively. The achieved results were nearly similar to that recorded by Mead *et al.* (1993), on the other hand lower results were obtained by Hamouda (2001). The high incidence of *Staphylococcus aureus* in chicken and chicken parts samples indicated that such organism was mainly associated with the skin of live birds. In general the high level of bacterial counts in most of the samples was probably a result of the defeathering process, which reportedly damages the intact carcass skin structure and exposes new surface for the attachment of bacteria (Kim and Doores, 1993 and Lindsay *et al.*, 1996). Also high level of bacterial counts of breast samples have been suggested as a results of fecal contamination from the broiler habit of laying on their breasts on the floor of broiler cages. Furthermore, Geornaras *et al.* (1998) stated that, the relatively high population of Enterobacteriaceae was indicative of fecal contamination which was probably responsible for an increase in the population of gram- negative isolation from all post scalded carcasses samples. Also, *Staphylococcus aureus* is well recognized for its ability to attach to rubber fingers of the defeathering machine and once established, to persist in the machine for months or even years (Gibbs *et al.*, 1978).

Incidence of the isolated Salmonella organism:

The incidence of *Salmonella* isolated from different examined samples was recorded in Table (2). The incidence was higher in whole carcasses, giblets and breast than that other parts (40, 30 and 30% respectively).

Regarding to whole carcasses results nearly similar to the results reported by Capita *et al.* (2003), while higher incidence was recorded by Antuneset *et al.* (2003). On the other hand, lower incidence was

achieved by Antown (2002) and Shaltout and Abd El-Aziz (2003). *Salmonella* had been also isolated from giblets in other studies where, Carraminana *et al.* (1994) recorded higher incidence. While, Hassouba (2005) reported lower incidence.

Concerning the incidence of *Salmonella* in breast samples, the obtained results were slightly higher than that achieved by Uyttendaele *et al.* (1999) and Buhre *et al.* (2000) while higher incidence of *Salmonella* recovered from breast samples were recorded by Kotula and Pandy (1995) than reported in this study.

Furthermore, the processing of breast fillets and part-boned portion of broiler which require a significant more handling of meat and lead to increase the risk of cross-contamination. This reflects the relative increase in the level of contamination of fillets and part-boned quarters being contaminated during processing. The serovar of the isolated *Salmonellae* were *Salmonella typhimurium*, *Salmonella kentucky*, *Salmonella infantis*, and *Salmonella enteritidis*. *Salmonella typhimurium* was isolated from chicken and chicken parts and giblets. *Salmonella typhimurium* was isolated from chicken, chicken parts and giblets by Antown (2002) and Hassouba (2005).

S. infantis was isolated by Hassouba (2005) from chicken and giblets samples and it was isolated from chicken and human stools of the workers in poultry slaughter house by Shaltout and Abd El-Aziz (2004).

Application of ozonated water:

Broilers carcasses were examined to determine the difference in microflora count before and after water and ozonated water immersion for 20 minutes (Fig. 1).

Results indicated that immersing the carcasses in water for 20 minutes reduced APC, coliforms count and *Staphylococcus aureus* counts by 0.84, 0.6 and 0.5 log cfu/g respectively. While, application of ozonated water for 20 minutes reduced APC and *Staphylococcus aureus* by more than 2 log cfu/g and reduced coliforms count by 1.4 log cfu/g. Moreover application of ozonated water for 30 minutes indicated more reduction in bacterial count (Fig. 2) where, APC and coliforms were reduced by more than 3 log cfu, while *Staphylococcus aureus* count was reduced by log 2.5 log cfu.

The present results indicated that immersion of carcasses in ozonated water for 30 minutes were more effective in reducing the bacterial population than that immersed in water for 20 min, 30 min and ozonated water for 20min. Our results were coincide with results obtained by Bailey *et al.* (1996) and Kim, 1998). While, Reagan *et al.*

(1996) and Bosilevac *et al.* (2005) recorded lower reduction level in APC. However, several studies were conducted to determine the disinfectant or antimicrobial effect of ozone in different food industries such as fresh vegetable processing (Kim, 1998 and Rodgers *et al.*, 2004), in fish (Campos *et al.*, 2005 and Gelman *et al.*, 2005) and on beef carcasses surface (Castillo *et al.*, 2003).

Fig (3) showed the effect of using ozonated water on *Salmonella typhimurium* in carcasses after 20 and 30 minutes immersion of carcasses. The populations of such organisms were reduced by 3.9 log cfu after application of ozonated water for 20 minutes. Whereas, *Salmonella typhimurium* was reduced by nearly 5 log cfu by application of ozonated water for 30 minutes.

Although immersion of water reduced *Salmonella* population in broiler carcasses, such reduction rate still limited compared by ozonated water reduction rate. Since carcasses immersed in water for 20 or 30 min recorded log 0.9 and log 1.3, reduction in *Salmonella* population while ozonated water reduced such organism by 3.9 log and after application for 20 min. whereas, it was reduced by nearly 5 log cfu by application of ozonated water for 30 min.

From the results it was obvious that the reduction of *Salmonella typhimurium* was greater after ozone treatment than after water treatment. Nearly similar results were recorded by Restaino *et al.* (1995); Bailey *et al.* (1996) and Rodriguez and Yousef (2005). While lower reduction rate of such organism were recorded by Fabrizio *et al.* (2002) and Castillo *et al.* (2003).

Hurst (1993) described a method for sanitizing food products that utilizes ozone bubbles. He argued that exposing food products to ozone-air bubbles effectively eliminate bacteria present on product surfaces. When poultry carcasses were spiked with *Salmonella typhimurium* and immersed into the water bath with ozone-air bubbles for 10 minutes, *Salmonella* count decreased by 2-3 log cfu.

The finding of the present study demonstrated ozone to be a suitable treatment process for reducing pathogenic microorganisms on broiler carcasses and in chiller water without sacrificing broiler carcass quality.

A number of factors still need to be considered prior to using ozone in poultry chillers or as a waste water treatment. These factors include equipment needs, toxicity optimal ozone to water ratios and government regulations. Major opportunity for using ozone in the

poultry industry was in the treatment of spent chiller water to a level suitable for recycling so, it can be used again.

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