The Consequences of Peroxyacetic Acid and Chlorine Decontamination Dips on the Native Pathogen and Spoilage Microbiota of Chicken Giblets, Plus their Shelf-life

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Abstract

THE current study aimed to determine the effects of chlorine (50 ppm) and triple-mixture of peroxyacetic acid (PAA, 300 ppm) dipping on the native spoilage and pathogen contamination levels and shelf-life of chicken giblets obtained from live poultry markets in Tukh City, Egypt, in comparison to Egyptian standardizing organization norms. In terms of shelf life, PAA- and chlorine-dipped giblets were hygienically acceptable, with APC values less than six log CFU/g until the ninth and sixth chilling-days, respectively. After six chilling days, the control giblets exhibited clear signs of decomposition; however, PAA and chlorine dipping delayed spoilage until the fifteenth and twelfth chilling days, respectively. Most of the hygienic indices of giblets dipped in PAA and chlorine did not differ statistically during the first chilling period; however, PAA eventually demonstrated a superior retardation impact on the growth curves of most native bacterial indices, including *Escherichia coli*. *Salmonella Enteritidis* was isolated from studied giblets with an average level of 2.8 log CFU/gram. PAA and chlorine dipping significantly impeded the growth curve of *Salmonella Enteritidis* in giblets, and it was not detectable for fifteen and twelve days, respectively. Finally, the combination of chilling (1± 0.5 °C) and tested antimicrobial dipping, specifically PAA, improved giblets hygiene indices and shelf-life after a five-minute. Therefore, although current findings suggest substituting PAA for chlorine, residual risks and associated environmental implications, as well as the evolution of antibacterial resistance, should all be considered prior to this step.

Keywords: giblets hygiene, shelf-life, decontamination efficacy; peroxyacetic acid, chlorine, *Salmonella*.

Introduction

Incidents impacting food safety are becoming more common and more widely reported worldwide [1]. A single incident that compromises food safety, such a foodborne illness outbreak, may prompt the recall or withdrawal of a food product and enforcement proceedings from the appropriate authorities at the food businesses involved [2]. Every year, the poultry sector faces a significant threat from foodborne pathogens like *Salmonella* and *Campylobacter* due to their link to foodborne illnesses [3]. Worldwide, non-typhoidal *Salmonella* is thought to be responsible for 93.8 million infections annually [4]. It is estimated that 80.3 millions of these infections are foodborne [5]. Salmonellosis is mostly caused by contaminated chicken meat; according to certain research, 25% of outbreaks of foodborne infections are linked to poultry [6,7].

The most popular place to buy fresh chicken meat in Egypt is live poultry stores; they are a staple of Egyptian culinary and retail culture and are accessible in most small towns and cities. Egyptian live poultry businesses’ best-selling items are raw chicken carcasses and their fresh byproducts, such as giblets. Carcasses, their subsequent cuts, and processed meat products are contaminated by pathogens from poultry microbiota, the slaughterhouse environment, and the equipment used during and after slaughter [8]. Since chicken processing is highly mechanized, different bacteria can contaminate poultry meat at different points in the manufacturing process where cross-
contamination can happen. Among the preharvest contamination risk factors that have been realized are transport crates, unfavorable ambient conditions, unclean worker practices, and bird-to-bird transmissible illnesses [3]. Poultry carcasses and of course giblets, get largely contaminated with harmful bacteria during processing because of fecal matter leaks during major processing phases. Cross-contamination has also been found as a significant danger during processing [9].

Poultry processing facilities should strive to reduce contamination events by implementing appropriate sanitary dressing procedures and using antimicrobial interventions during slaughter and carcass fabrication into parts, even if contamination of raw carcasses cannot be eliminated. Implementing adequate pathogen reduction performance requirements has the potential to reduce illnesses caused by the consumption of contaminated chicken products [10,11].

The most frequently utilized interventions worldwide and in the US poultry industry's chiller applications are peroxyacetic acid (PPA) and chlorine treatment. Moreover, the majority of previous investigations addressed the effects of these two disinfectants on chicken carcasses and cuts [10,12]; however, very few studies clarified the sanitary consequences on giblets. Prior Egyptian research has largely focused on the hygienic characteristics of raw chicken carcasses; however, only a few studies [13] have focused on the hygiene and pathogen control intervention of chicken giblets, which are also consumed in relatively close level to the chicken carcasses. More cooling and sanitary measures are needed with giblets to stabilize quality and lower native microbial growth curves. Thus, the objective of the current investigation was to assess the impact of triple-mixture of peroxyacetic acid (PPA) dipping and chlorine on the shelf-life and hygienic quality of chicken giblets purchased from live poultry markets.

Material and Methods

Experimental

Experiment management and approval.

The Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) at Benha University's Faculty of Veterinary Medicine authorized all protocols employed in this study under the number BUFVM 24-06-23.

Disinfectants preparation

Disinfectants and antibacterial solutions were independently developed in sanitized plastic food containers. Peracetic Acid dipping solution was made from a commercial triple-mixture of peroxyacetic acid, acetic acid, and H2O2 in a 15:30:10 ratio. 2 ml/litre of this triple-mixture was diluted with one litre of sterile distilled water, yielding a final concentration of 300 ppm (0.0300%) of peracetic acid, 600 ppm of acetic acid, and 200 ppm of H2O2. The PAA concentrations used in this study were ranges authorized by the US Department of Agriculture's Food [11]. A stock solution of food-grade chlorine was prepared by dissolving one tablet and serially diluting it to 50 ppm (0.005%). The control giblets were submerged in sterile distilled water.

Sample preparation and distribution.

On the day of the trial, fresh chicken giblets (without any antimicrobial treatment) were obtained from a local live poultry processing facility and transported to Benha University's Food Hygiene and Control Laboratory. To guarantee a random distribution of the initial spoilage and pathogen contamination load and to mimic production operations, all giblets were mixed in a cleaned container before being covered with sterile distilled water for 30 minutes at room temperature. Chicken giblets were aseptically transferred to a sterile steel sieve placed over another vessel to remove excess water. The chicken giblets were then randomly assigned to one of three treatments: control (sterile water), 0.005% chlorine or PAA-dipping (300 ppm, 0.030%, and 0.015% hydrogen peroxide). This resulted in a total of 36 giblet pieces per group, computed as three pieces per group multiplied by six checking days over two replications. For five minutes, chicken giblets were submerged in a variety of pre-made antimicrobial solutions in cleaned plastic food-grade containers. After their exposure times, the giblets were left to air dry on a sterile steel sieve. The control group only received sterile DW dips, and the same methods were followed for them. Once the giblets had dried, they were placed in a bag and stored in an incubator (Binder KB, BINDER GmbH, Tuttlingen, Germany) at 1± 0.5 °C. Days 0–15: The microbiological characteristics of the giblets were evaluated.

Microbiological assessment of chilled chicken giblets

Determination of aerobic plate count

The aerobic plate count (APC) in giblets samples was assessed in the same manner as for ground beef products [14]. Each sample was produced as a 10% homogenate, then serially diluted ten times, with 1 ml of each dilution inserted into two separate sterile Petri dishes. After that, the solidified inoculation plates were incubated for 72 hours at 30°C [15].

Determination of coliform count

One ml of previously prepared tenfold dilutions was inoculated into two independent sterile Petri dishes of Violet red bile agar at 37 °C for coliform enumeration in giblets [16].
**Determination of Staphylococcus count**

Offal’s *Staphylococcus* counts in were determined triple using the surface-plating method on the Baird Parker agar plate, as previously published for milk [17]. Using a sterile disposable spreader, one milliliter of each of the previously made serial dilutions was distributed over.

**Native E. coli growth curves monitoring**

Throughout the course of the experiment, Hektoen enteric (HE) agar was used for the direct screening of all *E. coli* strains.

**Native Salmonella detection, confirmation, and growth curves monitoring**

Giblets were tested for the presence of Salmonella species using a standard cultivation method recommended by ISO [18]. For direct Salmonella counting, one milliliter of 10% homogenate from three samples selected at random during the giblets randomization process prior to distribution was immediately streaked onto Hektoen enteric (HE) agar and XLD plates. The fundamental three steps for isolating *Salmonella* were also conducted concurrently to handle initial low number scenario: pre-enrichment on buffered peptone water for an overnight period at 37°C, enrichment on Rappaport Vassiliadis (RV) broth for an 18–24-hour period at 41°C, and final plating on HE agar plates (Condalab, Spain). Direct screening of the three samples on a selective isolation plate revealed positive *Salmonella* with characteristic colonies, which were later validated by MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France). *Salmonella* growth curves were then evaluated similar to other native populations, using HE agar and XLD plates 14

**Statistical Analyses**

SPSS Version 22 (SPSS Inc. Chicago, IL, USA) was used for data analysis. The effects of antimicrobial dipping, chilling periods (1, 3, 6, 9, 12, and 15 d), and their interaction on the giblets microbiological attributes were examined using general linear models (LMM), with giblets being treated as a random variable and antimicrobial dipping and chilling checkpoints as fixed effects. The means and standard errors of the results are displayed. The statistical model employed Tukey’s b multiple comparison test to assess the effects of antimicrobial dipping in comparison to the control, as well as to compare different monitoring point averages within the same group. Significant differences were defined as $P < 0.05$.

**Results**

Dipping giblets in chlorine and peroxyacetic acid immediately reduced APC development curves compared to the control from the first day ($P < 0.05$). After three chilling days, there was no significant difference in APC between the three groups, while the control group attained six log CFU/g ($P > 0.05$). On the sixth chilling day, control giblets exceeded the maximum allowable level of APC. However, treated giblets, especially those with PAA, stayed within the acceptable six log range ($P < 0.05$). Similar findings were detected after nine chilling days, albeit with greater numbers than the previous checkpoint (6th day). Significant differences were seen between dipped-giblets after twelve chilling days, with chlorine-treated giblets exceeding seven logs CFU/g ($P < 0.05$). At chilling completion, all control and disinfection dipped-giblets were comparable and exceeded seventh log CFU/g.

Both chlorine- and PAA-dipping had antimicrobial effects on coliform growth curves in giblets compared to the control on the first six chilling days, but the difference was not statistically significant ($P > 0.05$). On the second chilling half, PAA-treated giblets exhibited the lowest coliform growth curves than control and chlorine-treated giblets ($P < 0.05$). PAA-treated giblets had the lowest *Staphylococcus* growth curves, while the control had the highest. Chlorine-treated giblets had a medium level in between, which was comparable to the PAA effect across a few checkpoints ($P < 0.05$).

In comparison to the control and chlorine dipping, native *E. coli* was totally reduced by the PAA Antimicrobial action until the third chilling day ($P < 0.05$). Chlorine-treated giblets showed lower and/or equivalent *E. coli* growth curves than PAA-dipped giblets at the six-chilling point to the end of chilling period, whereas the control group displayed the greatest curves.

MALDI-TOF identified all suspected *Salmonella* isolates from the three giblets samples at the initial day of chilling as *Salmonella enterica* serovar Enteritidis (SE), with an average count of 2.8 log CFU/gram. *Salmonella Enteritidis* (SE) growth curves in dipped giblets were considerably inhibited by PAA, chlorine, along with chilling temperature, and could not be identified until twelve and fifteen days in chlorine- and PAA-treated giblets, respectively ($P < 0.05$).

**Discussion**

The current study aimed to assess the consequences of chlorine and peroxyacetic acid dipping on the hygienic quality and shelf-life of chicken giblets purchased from live poultry markets in Tukh City, Egypt, compared to the Egyptian standardization organization’s requirements. Chicken parts and giblets contain more harmful bacterial contamination level than full broiler carcasses due to increased handling and cross-contamination from other diseased parts or giblets [19]. The public health consequences, as well as the social and economic costs of food-borne illness, necessity safe food production and distribution from...
farm to table. Sanitary and hygienic procedures constitute an integral part of the fundamental concept of limiting microbial contamination during slaughter. The ultimate goal should be to minimize the microbial burden on the finished product while selecting abattoir technologies and running specific operations [20].

Numerous antimicrobial interventions have been studied for the safe manufacture of raw poultry products; nevertheless, most of these studies concentrated on whole chicken carcasses and their cuts [10,12], and in very few cases, on giblets. The only previous study that explored giblet shelf-life and prospective strategies to lengthen giblet shelf-life was undertaken in the United Kingdom. However, the cooling trials were conducted at 1 (±0.5) °C [13].

Chlorine has historically been utilized extensively in chicken processing as an antibacterial because of its low cost and low concentration needed to be effective. However, high temperatures, prolonged residence periods, large concentrations of organic matter, and pH variations all rapidly diminish the effectiveness of chlorine [10]. Peroxyacetic acid (PAA) is now the most applied antibacterial in the chicken business, replacing chlorine compounds and others. The United States Food and Drug Administration (FDA) approved the use of peroxyacetic acid (PAA) in raw poultry products (21 CFR 173.370), with a maximum concentration of 2,000 ppm of peroxyacids and 1,435 ppm of hydrogen peroxide depending on the application [11].

On six log CFU/g, the maximum authorized number of APC, PAA-, and chlorine-dipped giblets was acceptable until the ninth- and sixth-chilling days, respectively. While the control displayed visible spoiling by the sixth day, PAA- and chlorine-dipping delayed spoilage until the fifteenth- and twelve-chilling days, respectively. In terms of shelf-life, giblets kept at 1 °C were able to postpone spoiling for 12–14 days [13,21].

Compared to the control and, in certain cases, the chlorine treatment, the administered (300 ppm) PAA treatment considerably reduced most giblets native bacterial indices curves by one log CFU per gram. According to previous research, PAA treatment considerably reduced E. coli, coliforms, and aerobic plate counts (APC) compared to 0.003% (30 ppm) chlorine [22]. Nevertheless, current findings of coliforms, and APC is consistent with earlier study noticed insignificant differences between both treatment [19].

Between 2000 and 2017, there were twenty-eight foodborne outbreaks in the US linked to undercooked pâté, chicken liver, or both; eighteen of these outbreaks occurred between 2014 and 2016. Five of these twenty-eight outbreaks were caused by Salmonella, Campylobacter, or both [23]. The growth curves of SE were significantly suppressed by PAA, chlorine, and chilling temperature; were not detectable for twelve and fifteen days, respectively, in chlorine-treated giblets and PAA-treated giblets (P < 0.05). According to earlier research, all PAA concentrations (25, 100, and 200 ppm) in a poultry chiller, after 1 h, dramatically reduced Salmonella counts on inoculated carcasses when compared with the chlorine treatment (30 ppm), which is consistent with current findings [22]. Our earlier prevalence of Salmonella in chicken giblets was 27 %, which was distributed evenly, 33 %, between all chicken giblets groups [24]. Compared to earlier cross-sectional studies carried out in Egypt, which reported that the overall prevalence was 6.99% [25] and 11.1% [26], respectively, the current rate of Salmonella is much higher.

Hypochlorous acid and hypochlorite ions, which are forms of free accessible chlorine, are responsible for the antibacterial properties of aqueous chlorine solutions. Water pH must be lower than 7.0 to 7.5 to prevent the breakdown of hypochlorous acid into hypochlorite ions, which is more deadly to microorganisms than hypochlorite ions. While hypochlorite ions cause bacterial inactivation by breaking down the cell wall, hypochlorous acid exerts a destructive and nonselective oxidative action [20].

According to previous research [10], it has been revealed that a minimum of 400 ppm of a PAA-dipping was more efficient than chlorine for Salmonella eradication, however the current 300 PPM dosage was also effective. The justification of these results must consider additional variables such as exposure time, temperature, and pH. Though they might not be able to completely inactive pathogens, chilling and freezing have the potential to decrease their proliferation in cold preservation products. This should be taken into consideration when interpreting reduced growth curves of native populations, especially those of SE [27].

Despite the fact that chlorine and PAA are already effective decontaminants, their use in the poultry sector continues to face resistance, particularly in Europe [28]. The European Union (EU) has refused to authorize any therapies based on chlorine (19) and PAA because of their residues, such as 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), environmental impact, and the development of antimicrobial resistance. EU stakeholders consider that greater sanitary measures throughout production and processing are more suited to disease management than what they see as the United States’ excessive dependence on pathogen reduction treatments (PRTs) [28].

Conclusion

During the first chilling time, there was no statistically significant difference observed in most of the hygienic indices of giblets dipped in PAA and
chlorine. Nevertheless, PAA eventually showed a superior retardation impact on the growth curves of most native bacterial metrics, including *Escherichia coli*. From the assessed giblets, *Salmonella* Enteritidis was isolated, exhibiting an average level of 2.8 log CFU/gram. *Salmonella* Enteritidis in giblets was considerably inhibited by PAA and chlorine dipping, and it was obscured for fifteen and twelve days, respectively. Ultimately, within five minutes dipping, the combination of evaluated antimicrobial (PAA) and chilling (1± 0.5 °C) boosted the hygiene indices and shelf-life of giblets. Consequently, although current research suggests replacing chlorine with PAA, residual challenges and related impacts on the environment, along with the development of antibacterial resistance, should all be considered before taking this action.

*List of abbreviations*

peroxyacetic acid (PPA); *E. coli* (*Escherichia coli*).

*Acknowledgment*

Not applicable

*Conflicts of interest*

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TABLE 1. Shows the effects of chlorine and peroxyacetic acid (PAA) dipping on the microbiological quality and growth curves of native bacterial populations in giblets during 15 cooling days at 1±0.5.

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1APC, aerobic plate count; E. coli, Escherichia coli; SE, Salmonella Enteritidis; Dip, Dipping.
2Different superscript small letters (a, b, and c) within rows indicate that the dipping type has a significant effect on estimated parameters, while different superscript capital letters indicate significant effect of chilling time.
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