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Bacteriological evaluation of poultry meat treated with lactic acid and calcium chloride

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Abstract
Carcass contaminated during and after slaughter so, the current work was adopted to use some organic decontaminators to minimize bacterial load as lactic acid (0.5% - 1%) and calcium chloride (1% - 3%).
Chicken fillet divided into 7 groups, the first one was control, while remained 6 groups (2nd and 3 3d group) were dipped into lactic acid (0.5% - 1%) , the 4rd and 5th were dipped in calcium chloride (1% - 3%), 6th and 7th treated with mixture of (0.5% -1%) and (1% - 3%), of lactic and calcium chloride respectively. All samples were subjected to evaluate pH, aerobic plate count (APC), *Staphylococcus aureus* counts, and coliform counts at zero, 3rd and 5th days stored at 4°C.
In this current study, lactic acid show paleness in the treated samples in concentration 1% than 0.5%, while calcium chloride showed a little effect on the meat color, Chicken breast treated with calcium chloride showed best sensory quality including visual appearance and texture. The heights pH values were observed in control samples which increase from 6.07±0.21 , at zero day to 6.47±0.31 at 5th day of storage .The mean APC of control sample was decreased from 4.04 ± 0.69 at zero day to 2.28± 0.58, 2.03± 0.67, 3.26± 0.41, 2.97± 0.58, 2.14± 0.30 and 1.90± 0.16 log_{10} cfu/g . When treated with lactic acid 0.5, 1%, calcium chloride 1, 3% and in combination of lactic acid 0.5, 1% with calcium chloride 3% respectively at zero day of chilling at 4°c and reach to 4.57± 0.45, 3.07± 0.67, 2.76± 0.80, 4.25± 0.63, 3.46± 0.51, 2.60± 0.89 and 2.74±0.79 at the 5th day of chilling , the mean coliform count of control samples were 1.70±0.22 ±log_{10} cfu/g at zero day deceased after treatment with lactic acid 0.5% , 1% , calcium chloride 1% , 3% and combination of LA 0.5% , 1% with $Cacl_2$ to 0.94± 0.07, 0.69±0.12 , 0.74±0.35, 0.62± 0.36 , 0.64 ± 0.45 and 0.62± 0.31 log_{10} cfu/g at zero day respectively , while after chilling at 5th day there were reduction in the *coliform* count to 1.93± 0.03, 1.45±0.34, 1.73±0.21, 1.64±0.23, 1.57±0.29 and 1.19±0.11 log_{10} cfu/g respectively. The prevalence of *staphylococcus aureus* on chicken fillet have not been addressed, so this study proved that the greatest reductions were obtained by using combination of LA 1% and calcium chloride 3%.

Keywords: *Poultry meat, lactic acid, calcium chloride* **.**

Introduction

Poultry meat consumption is steadily increasing throughout the world. Therefore, increasing the microbial safety of poultry meat products is important in this context of increasing consumption and production. In fact, during and after slaughtering, the bacteria from animal microbiota, the slaughter house environment, and the equipment used contaminate the carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow and survive during food processing and storage. The resulting bacterial communities present in poultry meat include pathogenic species in addition to food borne pathogens which responsible for spoilage and may lead to large economic losses. Their growth and metabolic activity during shelf life leads to color, odour, taste or texture defects which are responsible for waste and losses of food products and have therefore an important

impact on the economy of the poultry meat production sector **(Rouger *et al*., 2017).**

The contamination of raw chicken with bacterial pathogens has important implications for public health. There is a great interest in reducing surface microbial contamination of carcasses meat, with particular regard to reduce the levels of pathogens **(Gonzalez *et al*., 2007).**

Raw meat, particularly poultry meat, remains an important source of human infection with pathogenic microorganisms. It can easily be contaminated with microorganisms because fresh poultry meat is very suitable for microbial multiplications. Meat has high water activity and high in nutrients and readily utilizable low molecular weight substances and source of carbon and energy by means of glucose, lactic acid, amino acids, creatines, metal and soluble phosphorus. As a result, fresh poultry meat is a suitable substrate for microbial multiplication **(Hinton, 2000).**

One approach to control the spread of these pathogens to the human population is to decontaminate the final product. Therefore, decontamination technologies are widely applied in meat and poultry slaughtering and processing plants under principles of Good Manufacturing Practice (GMP) and the Hazard Analysis Control Point (HACCP) system.

Various decontamination techniques have been used for the purpose including a strong and rapid decontamination action. Ideal decontamination should not have any residues that may be detrimental to the health of consumer. Furthermore, the treatment should not adversely affect taste, color, nutrition properties and appearance of the carcasses or meat. decontamination methods should be cheap, convenient to apply and not harmful. For chemical methods, all substances should improve the safety and shelf life of products by inactivating spoilage organisms as well as pathogens **(Van der Marel *et al*., 1988 and Hinton and Corry, 1999).** Microorganisms are settled in a wide range of environments; their genetic and physiological adaptability enable them to withstand numerous harsh and sometimes combined environmental factors. This ability to adapt and persist in harsh environments lies in how cells are able to sense and respond to environmental changes **(Moissl *et al*., 2016 and Esbelin and Hebraud, 2018).**

The bactericidal activity of chemicals is based on the disruption of cellular membranes, other cellular constituents and physiological cellular processes **(Loretz *et al*., 2010).** The application of organic acids has been investigated as a possible technology to reduce bacterial levels in many foods especially meat and meat products **(Lucera *et al*., 2010)**.

The mechanism of action of organic acids is depended on the ability of acid to permeate through the cell membrane an dissociate inside the bacteria causing a decrease in internal pH, which may interrupt ATP and RNA synthesis, DNA replication and cell growth **(Rojkovic *et al*., 2010).** Application of organic acids on meat surfaces is a common procedure; acid treatments are cheap, simple and fast and have shown clear efficiency **(Hinton and Corry, 1999).** Organic acids and their salts exert antibacterial activity. They have been traditionally used as food preservatives and are generally recognized as safe substances (GRAS) approved as food additive by EC, FAO/WHO and FDA **(Surekha and Reddy, 2000).**

Some organic acids, such as lactic acid have been extensively investigated as antimicrobial agents for use in meat, including poultry, to extend its shelf life and inhibit the growth of pathogens **(Mulder *et al*., 1987; El-khateb *et al*., 1993 and Conner *et al*., 1997).**

Lactic acid is a nontoxic, weak acid naturally produced in meat and offers the possibility of reducing spoilage of meat and meat products **(Cardenas *et al*., 2008)**, it is generally regarded as safe antimicrobial agent commonly used in meat and meat products for decontamination **(Kolua and Thelappurate, 1994).** Most applications of lactic acid, used for improving the quality of a variety of foods and for controlling microbial growth, are associated with the pH lowering effect **(Shelef, 1994).** The antimicrobial effect was attributed to both reduction of pH below the range required for microbial growth and metabolic inhibition due to undis-

sociated acid molecules **(Alvarado and Meckee, 2007).**

Chemical treatments have the potential to reduce microbial counts and may provide the basis for an effective intervention critical control point (CCP). They may also inhibit subsequent microbial growth therapy extending shelf life.
Using chemical decontamination methods does not only concern the antimicrobial effects but also the acceptable daily intake, because the dietary intake of lactic acid and calcium chloride is not limited, their use in meat products is favorable **(Mani-Lopez *et al*., 2012).**
Therefore, the sensory parameters (color and flavor) should be taken in consideration when assessing the suitability of individual chemical compounds as potential microbial decontaminants **(Hunt *et al*., 2012).**

The aim of this study was to evaluate the effectiveness of several concentrations of lactic acid and calcium chloride used separate and in combination together on the microbial growth in chicken fillet under aerobic conditions and stored at 4^{o}C.

Materials and Methods
Chicken fillets were obtained and investigated freshly, divided separately into 7 groups, the first one was control non treated while the remained 6 groups; two of which (the first and the second group) were dipped for 2 minutes in a freshly prepared solution of lactic acid 0.5% and1%, the 3rd and 4th were dipped in calcium chloride 1% and 3%. The 5Th group was treated with mixture of lactic acid (0.5%), and calcium chloride (3%). The 6th group was treated with mixture of lactic acid (1%) and calcium chloride (3%).

The pH value was determined according to **EOS (2006)**. It is one of the important factors to be considered in the application of chemical meat decontamination. The change of the pH value of poultry meat only slightly or even does not induce sensory attributes.
The sensory evaluation **(Cegielsks-Radziejewska *et al*., 2008**) include appearance odour and texture were carried out after treatment.

Microbiological analysis:
Samples preparation according to **ISO 6887-1/2017**
Samples were stomached for 30 s in diluent, serially diluted (1:9), plated onto appropriate media and subjected to the following examination:
1-*Total aerobic plate* count according to **APHA (2001).**
2-*Staphylococcus aureus* count according to **FDA (2001)**
3 -*Coliform* count according to **FDA (2002).**
The tested samples were stored in refrigerator at 4^{o}C for third day and fifth day for the same microbiological analysis. The experiments were replicated three times.
Statistical analysis:
A descriptive statistical analysis was performed to estimate the mean, minimum, maximum, Standard Error and Analysis of Variance (ANOVA) by Mean Analysis Procedure, **IBM SPSS. 20.0 (2011).**

Results and Discussion

Table (1). pH of treated chicken fillet (mean ± SD)

Treatment	zero day	3rd	5th
Control	6.07[a] ±0.21	6.23[a] ±0.25	6.47[a] ±0.31
Lactic acid 0.5%	4.96[b] ±0.56	5.23[b] ±0.55	5.43[b] ±0.51
Lactic acid 1%	4.67[c] ±0.55	4.93[c] ±0.55	5.40[b] ±0.40
Calcium chloride 1%	5.83[d] ±0.31	6.10[d] ±0.40	6.20[c] ±0.44
Calcium chloride 3%	5.73[e] ±0.31	6.03[d] ±0.40	6.10[c] ±0.36
Lactic acid 0.5%+ Calcium chloride 3%	5.40[f] ±0.30	5.53[e]±0.32	5.90[d] ±0.36
Lactic acid 1% + Calcium chloride 3%	5.03[g] ±0.25	5.20[b] ±0.26	5.87[d] ±0.15

There is no significant difference (P<0.05) between cells contain the same letter in the same column.

Table (2). Statistical analysis of aerobic plate count of treated chicken fillet (mean ± SD log cfu/g)

Treatment	zero day	3rd	5th
Control	4.04[a] ± 0.69	4.24[a] ± 0.63	4.57[a] ± 0.45
Lactic acid 0.5%	2.28[b] ± 0.58	2.64[b] ± 0.76	3.07[b] ± 0.67
Lactic acid 1%	2.03[c] ± 0.67	2.40[c] ± 0.72	2.76[c] ± 0.80
Calcium chloride 1%	3.26[d] ± 0.41	3.70[d] ± 0.18	4.25[e] ± 0.63
Calcium chloride 3%	2.97[e] ± 0.58	3.38[e] ± 0.47	3.46[f] ± 0.51
Lactic acid 0.5%+ Calcium chloride 3%	2.14[f] ± 0.30	2.49[f] ± 0.08	2.60[g] ± 0.89
Lactic acid 1% + Calcium chloride 3%	1.90[g] ± 0.16	2.39[c] ± 0.44	2.74[c] ± 0.79

There is no significant difference (P<0.05) between cells contain the same letter in the same column.

Table (3). Statistical analysis of coliforms count of treated chicken fillet (mean ± SD log cfu/g)

Treatment	zero day	3rd	5th
Control	1.70[a] ±0.22	2.06[a] ±0.23	2.82[a] ±0.12
Lactic acid 0.5%	0.94[b] ±0.07	1.25[b] ±0.37	1.93[b] ±0.03
Lactic acid 1%	0.69[b] ±0.12	0.99[b] ±0.01	1.45[ce] ±0.34
Calcium chloride 1%	0.74[b] ±0.35	1.13[b] ±0.22	1.73[bc] ±0.21
Calcium chloride 3%	0.62[b] ±0.36	0.99[b] ±0.05	1.64[bc] ±0.23
Lactic acid 0.5%+ Calcium chloride 3%	0.64[b] ±0.45	1.00[b] ±0.53	1.57[bc] ±0.29
Lactic acid 1% + Calcium chloride 3%	0.62[b] ±0.31	0.92[b] ±0.06	1.19[e] ±0.11

There is no significant difference (P<0.05) between cells contain the same letter in the same column.

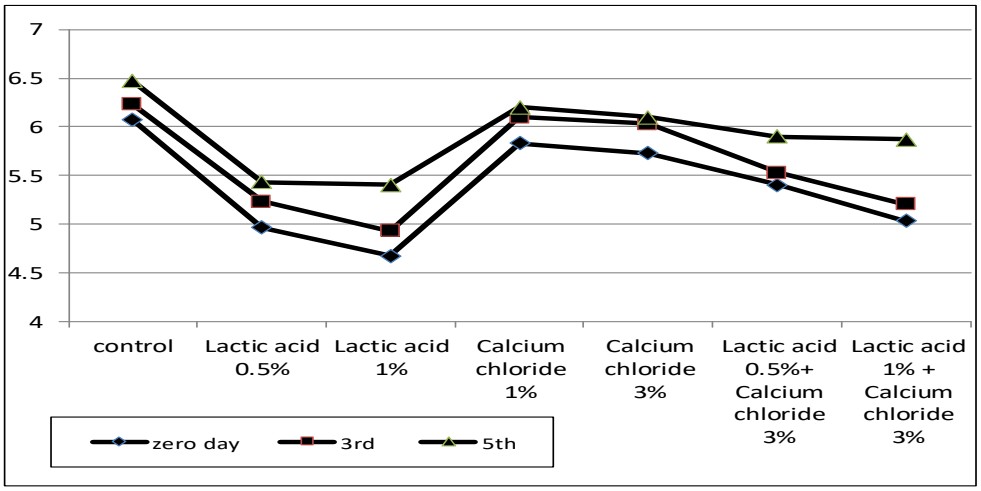

Fig. (1): pH of treated chicken fillet

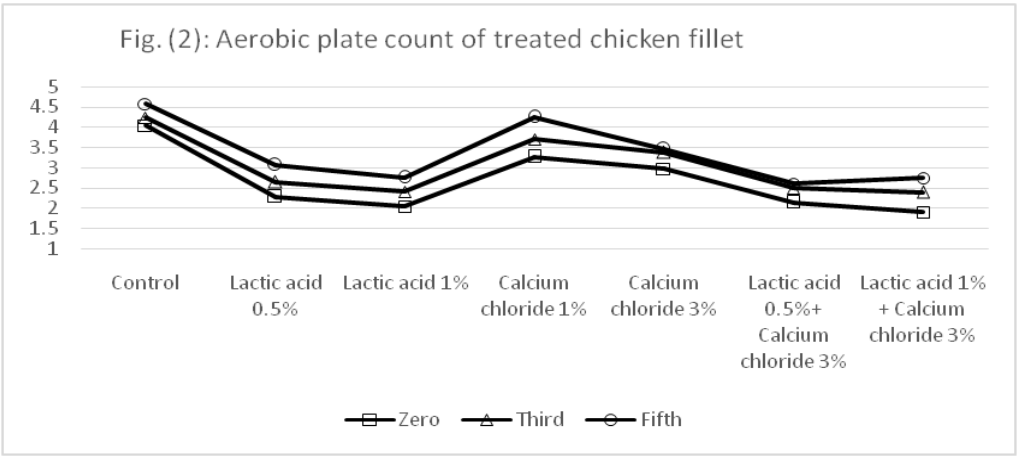

Fig. (2): Aerobic plate count of treated chicken fillet

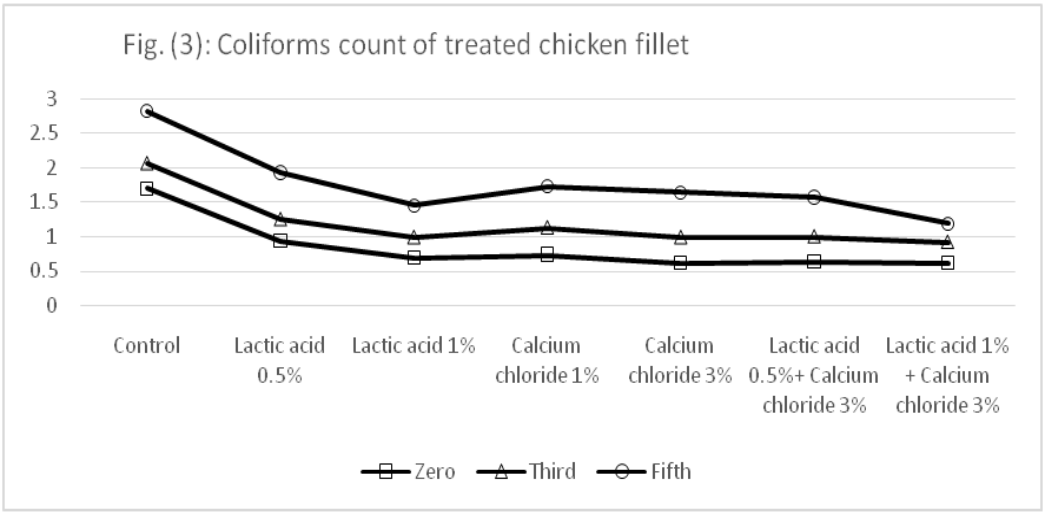

Fig. (3): Coliforms count of treated chicken fillet

Applying of lactic acid and calcium chloride in different concentration, alone or in combination into chicken fillet have a significant change on the microbial counts. As compared with untreated samples, these results were consistent with **(Kassem *et al*., 2017)** who concluded that all chemical treatments as food preservatives resulted in significant reductions in microbial population when compared to untreated controls.

Obviously, microorganisms have specific pH requirements for growth and a pH range within which their growth is possible. Therefore, even a small decrease in the pH by organic acids is sufficient to prevent the growth of many bacteria (**Stratford and Anslow, 1998)**.

The obtained results revealed that pH value of chicken breast was 6.07 in zero day. This similar to (**Corlett and Brown, 1980)** were (6.2-6.7). While, **ICMSF (1988)** illustrated that the pH of meat and poultry ranged from 5.6 to 6.4. The mean pH values of control and treated samples were shown in table (1) was significant increased in control samples during storage period due to accumulation of ammonia by bacteria, This agreed with **Gill (1983) and Alahakoon *et al*. (2014).**

The mode of action of organic acids in inhibiting microbial growth may be related to the ability of weak lipophilic acids as lactic acid to pass across the cell membrane in their undissociated form which dissociate within the cell and acidify the cell interior **(Shelef, 1994)**, In this respect, **Yaganza *et al*. (2009)** reported that several agents in salt solutions such as calcium chloride can inhibit the bacterial growth by elevating osmolality due to salt addition which may trigger the osmoregularity process causing increased maintenance metabolism and lead to reduce bacterial growth.

Changes in pH can influence the color of meat after treatment with chemicals **(Olivera *et al*., 2013)**, Therefore changes in sensory parameters should be taken into consideration. **Kassem *et al*. (2017)** illustrated that types and concentration of acid treatment influence the degree of meat discoloration due to difference in pH, in this current study, lactic acid showed paleness in the treated samples in concentration 1% than 0.5%, while calcium chloride showed a little effect on the meat color. Acids were used to improve texture of prepared meat products during storage **(Kjowski and Mast, 1993)**, while treatment with calcium chloride has become a popular method for meat tenderization because of the ease of its application and safe nature **(Gerelt *et al*., 2002)**. Chicken breast treated with calcium chloride showed best sensory quality including visual appearance and texture.

In the fifth day in the control chicken fillet the results of the three examined groups revealed increase of the initial microbial counts with mean 4.57 $\log_{10}$cfu/g for aerobic plate count and 2.82 $\log_{10}$ cfu/g for coliform as shown in tables (2, 3).

After application of lactic acid and calcium chloride in different concentration alone and in combination, there is reduction in the aerobic plate count in chicken fillet.

Treated samples with lactic acid 1% combined with calcium chloride 3% resulted in significantly lower microbial count as compared with other treatment, On zero day The mean APC for control was4.04 $\log_{10}$ cfu/g, (within permissible limit (10^5cfu/g) according to EOS (1090/2005),while after treatment with lactic acid 0.5, 1%, calcium chloride 1, 3% and in combination of lactic acid 0.5 with calcium chloride 3% and lactic acid 1% with calcium chloride the mean APC recorded 2.28± 0.58, 2.03± 0.67, 3.26± 0.41, 2.97± 0.58, 2.14± 0.30 and 1.90± 0.16 $\log_{10}$ cfu/g, respectively. At 3rd day of chilling in 4 C^{o} the control sample was recorded 4.24± 0.63 while the treatments were 2.64± 0.76,2.40± 0.72,3.70± 0.18, 3.38± 0.47, 2.79± 0.08 and 2.39± 0.44 $\log_{10}$ cfu/g respectively. At the 5th day of chilling the control sample showed 4.57± 0.45, the other treated samples were3.07± 0.67, 2.76± 0.80, 4.25± 0.63, 3.46±0.51, 2.90± 0.89 and 2.74±0.49 $\log_{10}$ cfu/g, respectively.

There were significant differences between APC of treated samples and control samples at zero, 3rd and 5th day of chilling where the count

of control sample were higher than 4 log_{10} cfu/ g while at the end of the chilling period the total plate count of the treated samples reached to2.74 log_{10} cfu/g in the combination of lactic acid 1% and calcium chloride 3%, while it was recorded 2.76 log_{10} cfu/g in lactic acid 1% treatment, this mean that the combination of calcium chloride and lactic acid was most effective antimicrobial treatment and increase of lactic acid concentration was beneficial treatment. Similarly, to that reported by **Alahakoon *et al*., 2014** and **Anang *et al*. (2010)** who reported that initial APC of chicken breast dipped in lactic acid decreased by 0.53 to 2.36 log_{10} cfu/g and **Eilers *et al*. (1994)** reported the antimicrobial effect of calcium chloride combined with lactic acid responsible for a significant reduction in microbial growth.

Bolton *et al.*, (2014). Established that mesophilic total viable counts (TVC) before treatment the average was 4.34 log_{10}cfu cm^{-2} of broiler carcasses after chemical treatment all TVC were significantly lower than control this inhibit microbial spoilage and extend shelf life. **Duan, *et al.* (2017)** reported similar result by using lactic acid 2% on chilled chicken carcass samples it causes reduction of 0.47-0.83 log_{10} cfu/cm^2 and 0.49-0.96 log_{10} MPN/cm^2 in TVC and total coliforms respectively.

Microbial spoilage occurs as a consequence of the growth and metabolic activity of spoilage bacteria. In most studies, the bacteria that detonate food spoilage have been considered those responsible for spoilage and, in some studies the criterion of microbiological acceptability total viable counts reaching 7 log_{10} cfu/g has been used to define spoilage. (**Rouger *et al*., 2017)**

In the current study, the mean fecal coliform counts in control samples were higher in the 5th day of chilling; its mean count was2.82 log_{10} cfu/g. The best reduction in the coliform count in the treated samples with lactic acid 1% combined with calcium chloride 3% recorded0.62±0.31,0.92±0.06 and1.19±0.11 log_{10} cfu/ g in zero, 3rd and 5th day respectively. The total *coliform* count in the examined samples varied from1.70±0.22 log_{10} in zero day to 0.62±0.31 log_{10} after combined treatment 1%, 3% and from 2.06±0.23 log_{10} to0.92±0.06 log_{10} in 3rd day and from2.82±0.12 log_{10} to1.19±0.11 log_{10} (table 3 and Fig 3,6). These results were confirmed the finding of **Yaganza *et al*. (2009)** who reported that the acidity or alkalinity of medium caused by addition of salt could have adverse effects on bacterial growth.

Staphylococcus aureus is associated with mucous membranes (nose and throat) and is commonly found on the skin and hair spread throw the air via coughing and sneezing and can contaminate meat also equipment and surfaces can be sources of contamination **(FDA, 2005)**, The prevalence of *Staphylococcus aureus* on chicken fillet have not been addressed.

These study revealed that application of organic acid (LA) and other chemical ($Cacl_2$) onto chilled chicken breast reduce the initial microbial counts , these results are similar to previous studies of **Kanellos and Burrel (2005),** reported that lactic acid between 1% and 3% concentration was optimal in regards to safety and product quality. Minimizing microbial contamination on poultry meat is dependent on the strict application of good farming practices and hygienic processing **(Bolton, *et al*., 2014).**

Lactic acid was reported to have broad bactericidal effects with advantages including lower toxicities and more stable forms to use in the field of chicken processing **(Burfoot and Mulvey 2011).**

Conclusion and Recommendations

This study concluded that lactic acid and calcium chloride can be used as antimicrobials for maintenance of good hygiene practices during chicken production. Lactic acid and calcium chloride offer several advantages as antimicrobials because they are Generally Recognized as Save (GRAS), have no limited acceptable daily intake, are low cost, easy to manipulate and effect minor sensory changes on products.

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