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Influence of Trisodium Phosphate on the Survival of *Salmonella* on Turkey Carcasses

Anita Mikołajczyk University of Warmia and Mazury in Olsztyn Poland

1. Introduction

Combating *Salmonella* in the environment is a difficult problem for those involved in livestock and food production. Table poultry are one of the main reservoirs of *Salmonella*. Contamination with *Salmonella* of up to 7% has been found in slaughter chickens immediately after stunning, while this figure has been observed to rise even to 48% in chickens prior to chilling (Mikolajczyk & Radkowski 2002a, 2002b). Despite numerous attempts to avoid secondary contamination by *Salmonella* on the slaughter line and during processing, there is an ongoing search for methods of rendering these bacteria harmless.

The number of chemical additives used in food processing is limited because of human health concerns, limits to solubility and governmental regulatory approval for direct application to foods. Further, consumers are demanding more "all natural", "organic", and "additive-free" foods, which limits the use of many chemical compounds.

In the search for methods of rendering *Salmonella* harmless, phosphates are worth considering. They are used by the poultry industry in many countries. Internationally, indepth research on the safe use of additives and their effects, suitability, and harmlessness to health is being done by the UNO through the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Advanced legislative work is also being done, independently of the JECFA, within the European Union. Within the EU, it is obligatory to use additives defined according to the EU numerical designation system, using the symbol E and an appropriate number. The same numbers are often used in many non-European countries, without the letter E.

In line with the numerical designation system of the European Union, sodium phosphates are distinguished by the symbol E 339, while trisodium phosphate is designated E 339 (E 339 iii) (Official Journal of the European Communities, 1995; Official Journal of the European Union, 2008).

On the basis of Union legislation, in Poland a Directive of the Ministry of Health (Directive of the Ministry of Health, 18 September 2005) allows the use of sodium phosphates (E 339) in foods for babies and small children. The maximum input of sodium phosphates used in production of food preparations intended for initial and further feeding of healthy babies is 1g/l in terms of P_2O_5 .

The lists of the International Food Additive Numbering System (INS), drawn up by the Codex Committee on Food Additives and Contaminants (CCFAC) feature synonyms and groups of additives. Some additives are multifunctional. Despite the international character of these lists, not all the additives are permitted to be used in individual countries. Individual country lists are subject to constant modification through removal, in other words, the introduction of prohibition of certain additives, and the addition of new ones. Sometimes substances previously denied approval are added to approved lists, following further research validation, as safe for health.

The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) publishes, in the Federal Register, regulations defining the process of approving the use of food product ingredients and sources of radiation in meat products, with the aim of enabling parallel control, carried out by the FSIS and the Food and Drug Administration (FDA), of applications to introduce new procedures and new additives and colourings to food items generally recognized as safe. The FDA publishes among its regulations a list (21 CFR - Code of Federal Regulations) of additives to food products and sources of radiation which are safe and appropriate for the production of meat and poultry products. The FDA regulations allow producers of ingredients and food products to determine, independently and using their own experts, whether a given substance is generally recognised as safe (GRAS - Generally Recognised as Safe). Substances with the statutory designation GRAS granted by internal experts are not included on the FDA list in CFR 21. The FDA sets out GRAS notifications for meat and poultry products in the GRAS Notification List, which it publishes on its own internet site. Substances permitted by the FDA regulations are approved for general use in food products (CFR 21, parts 172-180) or designated as substances generally safe for use in food products (CFR 21, parts 182 and 184).

Trisodium phosphate (TSP) is listed as approved in the CFR 21 and in the GRAS Notification, and may be used in meat and poultry production. "Trisodium phosphate is listed by the FDA as GRAS when used in accordance with good manufacturing practice." [Anon., 2005].

Trisodium phosphate is approved by the FDA as a food additive and is thus in the register entitled "Everything Added to Food in the United States" (EAFUS) [Anon., 2009].

2. The Influence of Solutions of Trisodium Phosphate (E 339 iii) Concentrations on *Salmonella* spp. in Microbiological Media and on Turkey Carcasses and Duration of Storage upon the Survival Rate of *Salmonella* in Turkey Carcasses

In the published literature, significant amounts of data relate to the widely accepted use of trisodium phosphate within the food industry and meat processing, justifying a study of the effects of trisodium phosphate on *Salmonella* during storage of carcasses. Hence the initiation of research aimed at determining the influence of trisodium phosphate on *Salmonella* present in microbiological media, on turkey carcasses and in samples from turkey carcasses kept for a period of 6 days, the maximum length of time before the meat reaches the consumer, may lead to an improved level of food hygiene and human health.

2.1 The Influence of Solutions of Trisodium Phosphate (E 339 iii) Concentrations on *Salmonella* spp. in Microbiological Media

2.1.1 Materials and methods

During stage one of the study, the influence of trisodium phosphate (E 339 iii) on *Salmonella* spp. was investigated in microbiological media. The following concentrations of analytically pure trisodium phosphate (E 339 iii) (Na₃Po₄) were added to nutritive agar were applied: 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 0.25%, 0.5%, 1%, 1.5%, 2.0%. *Salmonella* serovars studies included Enteritidis no. 33/66, *Salmonella* Anatum No. 30/93 and *Salmonella* Typhimurium No. 227/84, obtained from the Museum of bacterial strains of the National Veterinary Research Institute in Puławy in Poland.

The trisodium phosphate (E 339 iii) was sterilised using the Millipore filter (Millex 9P, 022 μ , Bedford), and added at appropriate concentrations to the medium at the temperature of 50°C. The *Salmonella* strains were inoculated into 9 ml of nutritive agar incubated for 24 hours at 37°C. This bacterial suspension was then used as an inoculum for further studies. Next, a ten-fold dilution of the culture was made and each serovar at each dilution was inoculated by spread plating on the nutritive agar without the TSP (negative control) as well as and on the nutritive agar supplemented with different quantities of trisodium phosphate (E 339 iii). Surface inoculation was applied. Plates were incubated at 37°C for 24 to 48 hours. Trials were replicated ten times and populations were averaged.

2.1.1 The Research Results

Table 1 and figure 1 detail results of the influence of various quantities of solutions of trisodium phosphate (E 339 iii) on Salmonella spp. in microbiological media. The average number of bacteria in control samples not supplemented with trisodium phosphate (E 339 iii) was 2.3×10^8 for S. Enteritidis, 7.25×10^7 for S. Anatum, and 2.6×10^8 for S. Typhimurium. Trisodium phosphate in agar medium at 1% concentration inhibited growth of Salmonella strains entirely. In the case of 0.5% concentration, the number of S. Anatum compared to the controls decreased by 3 logarithmic cycles, while S. Enteritidis and S. Typhimurium decreased by 4 logarithmic cycles. It was established that the addition of a concentration of up to 0.25% of sodium orthophosphate to an agar substrate had no substantial influence on the quantitative growth of any of the Salmonella examined. With a concentration of 1% trisodium phosphate, no increase in Salmonella in the substrate could be established.

Type of	Number of colonies [CFU/ml]										
Salmonella	Concentration of trisodium phosphate [%]										
spp.	0.00	0.01	0.02	0.03	0.05	0.1	0.25	0.5	1	1.5	2
S. Anatum	7.25×10^{7}	1.8×10 ⁷	6.0×10^{6}	8.0×10^{6}	8.7×10^{6}	1.2×10 ⁷	8.9×10 ⁶	2.0×10 ⁴	0	0	0
S. Enteritidis	2.3×10 ⁸	2.5×10 ⁷	2.2×10 ⁷	2.4×10 ⁷	2.4×10 ⁷	2.5×10 ⁷	2.6×10 ⁷	3.0×10^{4}	0	0	0
S. Typhimurium	2.6×10 ⁸	2.6×10 ⁷	1.9×10 ⁷	2.6×10 ⁷	2.8×10 ⁷	2.3×10 ⁷	1.9×10 ⁷	2.5×10 ⁴	0	0	0

Table 1. The growth of *Salmonella* spp. on agar substrate with an addition of trisodium phosphate (n=10)

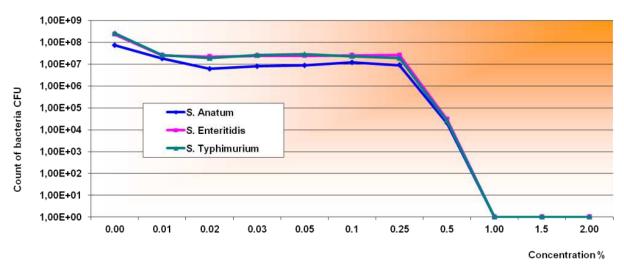


Fig. 1. The growth of *Salmonella* spp. on agar substrate with an addition of varying concentrations of trisodium phosphate

On the basis of the results displayed in table 1 and figure 1, it can be concluded that the addition of sodium orthophosphate had an influence on the survival of *Salmonella* in agar substrates. While the inhibiting influence of sodium orthophosphate in bacterial substrates was high, this compound may prove useful for destruction of *Salmonella* on poultry carcasses.

2.2 The Influence of Trisodium Phosphate (E 339 iii) on *Salmonella* Enteritidis on Turkey Carcasses

2.2.1 Materials and methods

This stage of the studies concerned analysis of the influence of trisodium phosphate (E 339 iii) on *Salmonella* Enteritidis present in elements of turkey carcasses.

Tests were conducted on 236 samples of turkey breast purchased from poultry processing plants. After delivery to the laboratory, the material was kept in a refrigerator at 4°C, and next used for preparation of 25 g samples for further analysis. No *Salmonella* spp. was detected when random samples (20% of all turkey breasts purchased) were examined for *Salmonella* spp.). *Salmonella* Enteritidis No. 33/66 was first inoculated in nutritive broth and incubated at 37°C for 24 hours. Turkey breast samples were then inoculated with 0.05 ml of the 24-hour broth culture of *S*. Enteritidis diluted to from 10⁻⁴ to 10⁻⁸ and initial inoculum of test samples was determined for each test series. The bacterial suspension was delicately spread with a special wide loop over the widest area possible. After inoculation with the bacteria, each sample was held for 20 minutes in a refrigerator at 4°C to fully dry the suspension. Next, each sample was transferred to a sterile beaker with 250 ml of a 1%, 2.5%, 5% or 10% trisodium phosphate (E 339 iii) solution for 15 minutes. Among the methods recommended for detection of *Salmonella* spp. on poultry carcasses, pluck and products the method given in ISO 6579, 1993; PN-ISO 6579, 1998 was applied.

Following 15 minutes of treatment in a trisodium phosphate (E 339 iii) solution, each sample was moved to a sterile beaker and covered with 225 ml of buffered peptone water (BPW, CM 509, Oxoid Basingstoke Hampshire, UK), and incubated at 37°C for 20 hours. Selective growth was achieved on SC medium (SC, 0 687–17-1, Difco Laboratories Detroit MI, USA),

Müller-Kauffman medium (MK, CM 343, Oxoid Basingstoke Hampshire, UK) and Rappaport-Vassiliadis medium (RV, CM 669, Oxoid Basingstoke Hampshire, UK) while the further culturing was done on brilliant green and phenol red agars (BGA, CM 329, Oxoid Basingstoke Hampshire, UK) on BSA medium (BSA, 00 73-01-1, Difco Laboratories Detroit MI, USA) and on XLD agar (XLD, CM 469 Oxoid Basingstoke Hampshire, UK). Colonies typical and suspected of belonging to *Salmonella* spp. were identified by serological and biochemical methods. Biochemical characteristics of *Salmonella* spp. were determined using API Test 20 E. Serological types were determined on the basis of the Kauffmann-White classification scheme as proposed by Popoff and Le Minor (1997) using the sera produced by the National *Salmonella* Centre.

Turkey breast samples inoculated with *Salmonella* spp. immersed in sterile water for 15 minutes were used as positive controls. Each variant of the experiment was done in ten repetitions.

2.2.2 Research results

Table 2 clearly demonstrates that inactivation of *Salmonella* on turkey carcasses is inversely proportional to the population of the original inoculum.

When 10 colony-forming units of *Salmonella* were inoculated onto the surface of a turkey carcass parts and treated for 15 minutes in 1%, 2.5%, 5% and 10% sodium orthophosphate, or either treated at 5% and 10% for 15 min., no *Salmonella* was detected. When 1% and 2.5% of sodium orthophosphate was used, there was a reduction in the number of samples in which *Salmonella* were found in relation to the control samples at both times as well as at a surface contamination of 10³ colony-forming units and treatment for 15 minutes, , in a 5% or 10% solution. Where a surface contamination with 10⁴ colony-forming units was applied to turkey meat immersed in the water solutions of the chemical substances in the study, their influence on detectability of *Salmonella* could not be confirmed (table 2).

Concentra-	Treatment	Salmonella Enteritidis nr 33/66							
tion	time	dilutions (inoculum)							
[%]	[minutes]	4	5	6	7	8			
		Number of positive results							
0	15	10	10	10	9	0			
	45	10	10	10	9	0			
1	15	10	10	9	0	0			
	45	10	10	5	0	0			
2.5	15	10	10	4	0	0			
	45	10	10	2	0	0			
5	15	10	9	0	0	0			
	45	10	7	0	0	0			
10	15	10	5	0	0	0			
	45	0	0	0	0	0			

Table 2. Number of samples from elements of turkey carcasses treated with trisodium phosphate, in which *Salmonella* Enteritidis was detected (n=10)

This study confirms the efficacy of the inhibiting influence of sodium orthophosphate on *Salmonella* on poultry carcasses. The unfavorable effect of this compound in relation to *Salmonella* was greater in bacterial substrates than on poultry carcasses.

2.3. The Influence of Trisodium Phosphate (E 339 iii) on survival of *Salmonella* Enteritidis on Turkey meat stored at a temperature of 4°C for 2, 4 & 6 Days

2.3.1 Materials and methods

Research was carried out on 192 samples of turkey breast purchased from poultry processing plants. Each sample was divided into two parts. One part was checked for the natural occurrence of *Salmonella*, while the other was inoculated with the relevant strain.

S. Enteritidis strain no. 33/66 was stored on agar strips in a refrigerator at a 4°C, then seeded into a nutrient broth and incubated at 37°C for 24 hours. After incubation, 10 ml of the broth were decanted into 4l of diluent consisting of: 1g peptone, 8.5% sodium chloride NaCl, and 1,000 ml distilled water, in which the turkey breast samples were immersed. After 5 minutes, the samples were extracted, drained for 2 minutes, placed on specially prepared sterile trays with drainage grids, and kept in a refrigerator at a temperature of 4°C for 20 minutes. The initial inoculum level of control samples was determined in each series of investigations. The samples were then transferred to sterile beakers containing 250ml of 1%, 2.5%, and 5% solutions of analytically pure (E 339 iii) trisodium phosphate (Na₃PO₄) for 15 minutes. The control material in this experiment consisted of breast samples inoculated with *Salmonella* and immersed in sterile water for 15 minutes. These were examined directly without decontamination, the number of bacteria being taken as the inoculum.

Swabs were taken from the exterior and interior surface of the turkey breasts using sterile tampons and templates. A stainless-steel template with a 25cm² aperture was applied to each surface examined. Two swabs were taken, one from the exterior, the other from the interior surface, the total surface area being 50 cm². The tampons with the swabs were placed in flasks with glass beads in 50ml of diluting fluid and shaken energetically for about 2 minutes. This achieved an initial dilution in which 1ml of fluid corresponded to 1cm² of surface examined. The fluid was then diluted tenfold and the level of Salmonella determined using the Most Probable Number method (ISO 7218, 1996). To achieve this, 1ml each of the initial suspension and its subsequent tenfold dilutions were decanted into 3 parallel testtubes of buffered peptone water (BPW, CM 509, Oxoid, Basingstoke, Hampshire, UK). These were incubated at 37°C for 20 hours and then re-decanted onto a selenite cystine substrate (SC broth), a Müller-Kaufman substrate, and a Rappaport-Vassiliadis substrate. After 24 hour incubation at 41.5°C (RV) and 37°C (MK & SC), an inoculation was made into an agar substrate with brilliant green and phenol red (BGA, CM 329, Oxoid, Basingstoke, Hampshire, UK), a bismuth-sulphite substrate (BSA 00 71-01-1, Difco Laboratories, Detroit Mi., USA), and a substrate with xylose, lysine, and deoxycholane (XLD, CM 469, Oxoid, Basingstoke, Hampshire, UK). A reading of the most probable number of Salmonella was made using the Hoskins table.

The research was carried out in line with methods set out in PN-ISO 6579, 1998; PN-A-82055-3, 1994; ISO 6579, 1993; ISO 7218, 1996. Each turkey breast was quantitatively examined for *Salmonella* immediately and after 2, 4, and 6 days of refrigerated storage at 4°C. This process was repeated six fold for each experimental variant.

The numerical material collected in the experiment was processed statistically using Student's T – test and correlation analysis. Correlation analysis was carried out on numbers expressed as logarithms.

2.3.2 Results and discussion

Table 3 and figure 2 show the changing numbers of *Salmonella* during storage of turkey meat samples at 4°C. The average initial contamination of the turkey samples amounted to 2.3x 10³ *Salmonella*. Following immersion in water, an average of 4.3x 10² *Salmonella* were recovered, which was used as the inoculum. The initial inoculum of *Salmonella* Enteritidis amounted to 10² colony-forming units per 1 cm² surface area of the turkey meat sample. Directly after application of the selected concentrations of trisodium phosphate (E 339 iii) solutions, it was possible to confirm their influence on the number of *Salmonella*, reflected by a substantial decrease in the number of colonies.

A concentration of 2.5% and 5% solution of (E 339 iii) trisodium phosphate caused a reduction of Salmonella on a poultry meat sample by 1 logarithmic cycle. It was also established that during storage in a refrigerator at a temperature of 4°C the number of Salmonella in the meat samples decreased. After 6 days, this reduction was greatest where a 5% solution of trisodium phosphate (E 339 iii) was used, amounting to 2 logarithmic cycles. A similar reduction of 2 log cycles was observed in the case of meat samples immersed in sterile water after 2 days of storage treated with a 5% solution. In the remaining variants of the experiment where a 5% solution of trisodium phosphate (E 339 iii) was used, S. Enteritidis was reduced by 1 logarithmic cycle. A 2.5% solution of trisodium phosphate (E 339 iii), in the case of samples immersed in sterile water, reduced Salmonella on the meat samples by 1 logarithmic cycle both directly after contamination and after 2 and 6 days of storage. Nevertheless, after 4 days of storage, S. Enteritidis increased in numbers within the same logarithmic range. Where a 1% solution of trisodiumphosphate(E 339 iii) was used, S. Enteritidis increased in numbers within the same logarithmic range both directly after contamination and after 2 days of storage. During the remaining days of storage, the number of bacteria rose by 1 logarithmic cycle in the case of samples immersed in sterile water.

Carlostanasa	Concentration [%]		ays' sto	Dependence of number of bacteria on duration of storage				
Substances examined		Directly after contami-nation	2	4	6	Correlation coefficient	Level of significance	
		Nun	nber of b	Г	P			
Control	0	2.3×10 ³	4.3×10 ²	2.3×10 ²	2.3×10 ²	- 0.89	> 0.10	
Water	0	4.3×10 ²	2.4×10 ²	9.3×10 ¹	4.3×10 ¹	- 0.99	0.01	
	1	1.2 ×10 ²	1.2 ×10 ²	2.4×10^{2}	2.3 ×10 ²	0.88	> 0.10	
Na ₃ PO ₄	2.5	9.3 ×10 ¹	4.1 ×10 ¹	9.3 ×10 ¹	2.3×10 ⁰	- 0.76	> 0.10	
	5	2.3×10 ¹	2.3×10 ⁰	2.3×10 ⁰	3.6×10 ⁻¹	- 0.94	0.05	

Table 3. The influence of trisodium phosphate on survival of *Salmonella* Enteritidis on turkey carcass elements stored at 4°C for 2, 4 & 6 days (n=6)

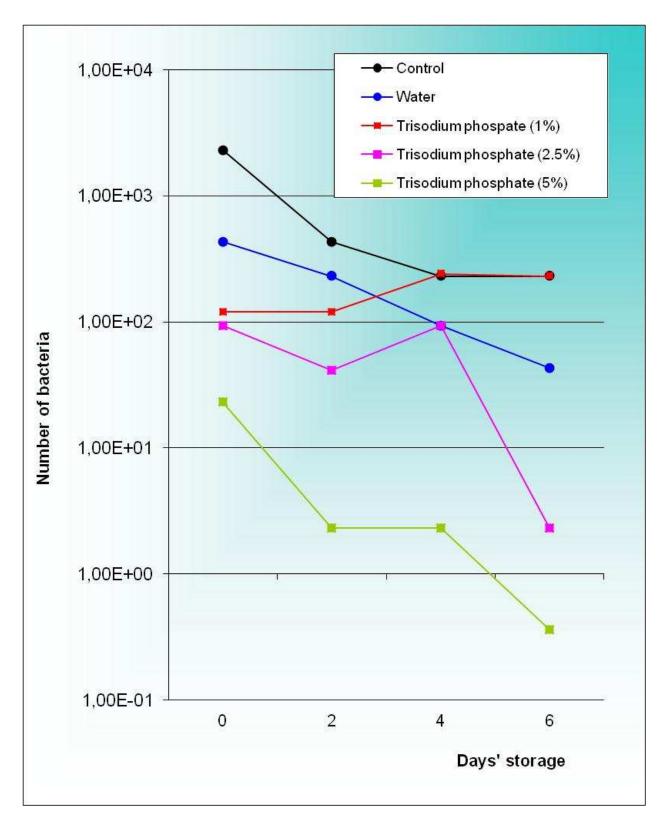


Fig. 2. The influence of sodium phosphate on survival of *Salmonella* Enteritidis bacteria on elements of turkey carcasses stored at 4°C for 2, 4, & 6 days

After slaughter, poultry carcasses with an internal muscle temperature of around 40°C must be subjected to chilling to reach a temperature of 4°C within approximately 2 hours or less, in order to extend the meat's keeping period and inhibit the growth of microorganisms. If poultry carcasses are contaminated with *Salmonella* during this production cycle, current technologies are not sufficient to render the pathogen harmless. Air temperatures in the range of 0°C to -1°C do not render *Salmonella* harmless and carcasses contaminated with them may be delivered for sale. The time before a carcass reaches the consumer after chilling is variable and depends on many factors. The producer, on the basis of research into storage, defines the date up to which fresh poultry is fit for consumption.

In the author's research, the influence of various concentrations of solutions of trisodium phosphate (E 339 iii) upon *Salmonella* was determined in conditions as near to natural as possible, in other words, using poultry meat originating directly from poultry processing plants, not subjected to any processes intended to eliminate accompanying microflora in the laboratory, and stored in conditions with a constant temperature of 4°C.

Hwang and Beuchat (1995), studying the effectiveness of reduction in the number of *Salmonella* on chicken skins under the infuence of trisodium phosphate (E 339 iii), confirmed a reduction in the number of these microorganisms only following immersion for 30 minutes at a temperature of 25°C in a 1% solution of NA₃PO₄. Bender and Brodsky (1990) observed that neutralization of the bacteria under the influence of a 10% solution of potassium phosphate for 15 minutes was incomplete, probably because of the formation of a coating of fat on the poultry skin. According to Giese (1992) this observation should enable the removal of bacteria from the surface of carcasses.

The mechanism by which trisodium phosphate (E 339 iii) kills *Salmonella* is not fully known. As indicated by Giese (1992 &1993), the effectiveness of using this medium is linked not only with its strong antibacterial properties but also with the possibility of removing the thin lipid layer from the surface of poultry skin.

Benedict et al. (1991) confirmed that the high index of the bacterial suspension's adhesion to the skin is achieved thanks to immersion of the carcasses in it. The serotype of the *Salmonella* and the temperature of the bacterial suspension do not influence the adhesion of microorganisms on the skin (Conner & Bilgili, 1994). Cell structures such as fimbriae or cilia are essential in the mechanism of adhesion to the skin (Dickson, 1992; De Graft-Hanson & Heath, 1990). A very important role is played by the duration of contact between the bacterial suspension and the skin (Conner & Bilgili, 1994). Directly after applying the relevant bacterial culture to the skin, samples should be kept for an appropriate period with the aim of obtaining better adhesion of bacteria to the skin of the carcasses. According to Conner and Bilgili (1994), the optimal time needed for *Salmonella* to settle and attach themselves on the skin is 10 minutes if an inoculum of 10⁴ is used. Where a lower inoculum is used, the time should be extended to 20 minutes with an inoculum of 10³ and 30 minutes with one of 10².

Salmonella which are firmly attached to the skin ("firmly attached cells") are significantly more resistant to the effect of chemical media than those which have not succeeded in settling firmly but are loosely attached to the skin ("loosely attached cells") (Lillard, 1989a, 1989b; Tamblyn et al., 1997). Bailey et al. (1986) observed a 90-96% reduction in S. Typhimurium as a result of a 3.5-second spray using sodium hypochlorite at 20-40 ppm.

Such methods often reduce but seldom eliminate *Salmonella* on poultry carcasses because they are ineffective in relation to bacterial cells settled on or firmly attached to the skin. Hence, there is a need to test media which neutralises *Salmonella* that are firmly attached to the skin (Tamblyn & Conner, 1997; Conner & Bilgili, 1994).

The critical point in research on neutralisation of *Salmonella* is the fact that these bacteria can be firmly attached to the skin, especially when carcasses are in the initial phase of the production process. It can even be the case that *Salmonella* become irreversibly attached to the skin. In view of this, it might be the case that no currently utilized antimicrobial will be fully effective (Conner & Bilgili, 1994).

The next problem in assessing the effectiveness of media inactivating *Salmonella* is the fact that despite the use of various techniques in rinsing, washing, and multiple re-rinsing of carcasses, not all bacteria can be removed (Izat et al., 1991). Lillard (1989b) confirmed that removal of bacteria attached to poultry skin is very difficult, though repeated rinsing can lead to removal of large numbers. In many publications (Conner & Bilgili, 1994; Izat et al. 1991) it is stated that the removal of *Salmonella* is based on multiple rinsing; nevertheless, it is apparent that not all cells can successfully be removed.

3. Conclusion

- 1. The inhibiting influence of sodium orthophosphate in bacterial substrates was high. The unfavorable effect of this compound in relation to *Salmonella* can also be applied to poultry carcasses.
- 2. Among the various concentrations of solution used for neutralizing *Salmonella* on elements of turkey carcasses stored at a temperature of 4°C for 6 days, the greatest effect was shown by a 5% solution of trisodium phosphate.
- 3. With samples treated with a 1% solution of trisodium phosphate (E 339 iii), stored for 6 days, the number of *Salmonella* did not undergo any substantial changes, still remaining at the same level.
- 4. In the case of 2.5% and 5% solutions of trisodium phosphate (E 339 iii), it was noticed that, along with an extension of the storage period, their limiting influence on the number of *Salmonella* in turkey carcasses increased.

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