

THE SURVIVAL OF *SALMONELLA* SPP. IN RELATION TO EXPOSURE TO LACTIC ACID AND THE STORAGE TIME OF TURKEY CARCASSES

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Key words: *Salmonella*, lactic acid, storage, turkey carcasses.

Abstract

The studies aimed at determining the influence of lactic acid on *Salmonella* spp. during storage of turkey carcasses' samples for 2, 4 and 6 days. The initial average contamination of turkey carcasses' elements with *Salmonella* spp. was $2.4 \cdot 10^3$ bacteria. Following the immersion in water in average $4.3 \cdot 10^2$ *Salmonella* spp. cfu was recovered and that number was assumed as the inoculum. The number of *Salmonella* spp. decreases during storage of turkey carcasses' samples in the refrigerator at 4°C. Compared to elements of carcasses immersed in sterile water the largest reduction, by two logarithmic cycles was recorded after 2 days of storage of samples treated with 1% lactic acid. In case of the other variants of the experiment when 1% solution of lactic acid was applied *S. Enteritidis* grew in numbers within the same logarithmic range. Compared to the samples immersed in sterile water, 2% lactic acid caused reduction in the number of *Salmonella* spp. on elements of poultry carcasses by one logarithmic cycle both immediately after contamination and after 2 and 6 days of storage; unfortunately after 4 days of storage *S. Enteritidis* grew in numbers that were within the same logarithmic range.

During storage of the turkey samples tested at 4°C for 2, 4 and 6 days, the numbers of *Salmonella* spp. decreased. That decrease compared to samples immersed in sterile water was the largest after 2 days of storage after application of 1% lactic acid.

WPLYW KWASU MLEKOWEGO I CZASU SKŁADOWANIA TUSZEK INDYCYZCH NA PRZEŻYWALNOŚĆ PAŁECZEK *SALMONELLA*

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Abstrakt

Celem badań było określenie wpływu kwasu mlekowego na pałeczki *Salmonella* podczas przechowywania próbek tuszek indyjskich przez 2, 4 i 6 dni. Średnie wyjściowe zanieczyszczenie bakteriami elementów tuszek wynosiło $2.4 \cdot 10^3$. Po zanurzeniu w wodzie izolowano średnio $4.3 \cdot 10^2$ pałeczek *Salmonella* – liczbę tę przyjęto za *inoculum*. Zmniejszyła się ona podczas przechowywania próbek w lodówce w temperaturze 4°C. Największy spadek o dwa cykle logarytmiczne, względem elementów tuszek zanurzonych w jałowej wodzie, odnotowano po 2 dniach przechowywania próbek potraktowanych 1% kwasem mlekowym. W pozostałych wariantach doświadczenia po zastosowaniu 1% roztworu kwasu mlekowego liczba *S. Enteritidis* rosła w liczbach mieszczących się w tym samym przedziale logarytmicznym. W porównaniu z próbkami zanurzonymi w jałowej wodzie 2% kwas mlekowy powodował redukcję pałeczek *Salmonella* na elementach tuszek drobiowych o jeden cykl logarytmiczny zarówno bezpośrednio po kontaminacji, jak i po 2 i 6 dniach składowania. Niestety po 4 dniach przechowywania liczba *S. Enteritidis* rosła w liczbach mieszczących się w tym samym przedziale logarytmicznym. W trakcie przechowywania badanych próbek indyjskich w temperaturze 4°C przez 2, 4 i 6 dni zmniejszyła się liczba pałeczek *Salmonella*. Spadek ten, w porównaniu z próbkami zanurzonymi w jałowej wodzie, był największy po 2 dniach składowania, po zastosowaniu 1% kwasu mlekowego.

Introduction

Salmonellosis is a serious epizootic and epidemiological problem. Detailed analyses of disease cases show that next to hen's eggs, poultry meat is the main cause of food poisoning cases. Slaughter poultry is the main reservoir of *Salmonella* spp. In slaughter chicken and turkey immediately after stunning contamination with *Salmonella* spp. bacteria involving up to 7% of them was found while before chilling it increased to even as much as 48% in case of chicken (MIKOŁAJCZYK, RADKOWSKI 2001a, MIKOŁAJCZYK, RADKOWSKI 2001b, MIKOŁAJCZYK, RADKOWSKI 2002b, MIKOŁAJCZYK, RADKOWSKI 2002c). The above results obtained in Poland are correlated with the studies conducted by the USDA Food Safety and Inspection Service that indicate that 4–5% of broilers brought for slaughter were infected with *Salmonella* spp. while poultry leaving the slaughterhouse was contaminated in 35–36% (LILLARD 1990). This is the consequence of post-slaughter contamination, which occurs at every processing step. As a consequence, there is high probability that poultry reaching the consumer will be contaminated with *Salmonella* spp. to an even higher extent. In 55% of poultry carcasses from shop shelves tested by BYSTROŃ at al. (2004) *Salmonella* spp. was recovered. *S. Enteritidis* was the dominating serological strain. The high percentage of poultry carcasses in retail outlets contaminated with *Salmonella* spp. indicates the direct risk to the consumers.

The number of *Salmonella* spp. on poultry carcass purchased is from 1 to 20 cells (FEHLHABER 1996). Although that number is low there is always the risk of *Salmonella* spp. growth in the meat. Whether symptoms of disease appear and what the development of the disease would be depends on the number of *Salmonella* spp.

Disease development in humans requires infecting the alimentary system with a larger number of *Salmonella* spp. cells than in case of the typhoid fever or paratyphoid fever, e.g.: *S. Newport* $1.0 \cdot 10^5$, *S. Bareilly* $1.0 \cdot 10^5$, *S. Enteritidis* $1.0 \cdot 10^5$, *S. Derby* $1.5 \cdot 10^6$, *S. Anatum* $5.0 \cdot 10^6$, *S. Meleagridis* $5.0 \cdot 10^7$, *S. Gallinarum* $1.3 \cdot 10^9$ (DUGID et al. 1991). It should be added that in frequent cases low numbers of *Salmonella* spp. were recovered from the food that was the cause of poisoning. This applied mainly to cases in small children and the elderly. The number of *Salmonella* spp. cells in a poultry carcass that is a threat to human health depends on the person's age and health condition, i.e. the individual biological immunity.

Despite numerous attempts at avoiding secondary contaminations with *Salmonella* spp. on the slaughter line and during processing the search for methods of eliminating the bacteria is still searched for. For many years, in the USA, which is the largest in the world chicken producer, sodium hypochlorite has been added to water immediately prior to the chilling process to destroy *Salmonella* spp. cells. Sodium hypochlorite is considered safe and as a consequence chicken subjected to treatment with it are also found in shops within the European Union. However, as sodium hypochlorite at higher concentrations causes colour and flavour change, search for another method of eliminating those bacteria continues.

The number of chemical additives applied in food processing is limited as a consequence of their negative influence on human body and difficulties with solubility and direct application. Additionally, eco-focused lifestyle of the consumers forces use of only those chemical substances that appear in the environment naturally in food processing.

In search for methods of eliminating *Salmonella* spp. attention has been focused on a large group of organic acids and their salts that are commonly considered safe. Polish food industry allows use of the following organic acids as food additives: lactic, acetic, citric, tartaric and their salts (Rozporządzenie Ministra Zdrowia... 2008).

Lactic acid is a natural component of many food products such as sauerkraut, pickled cucumbers, cured sausages or yoghurt. It finds wide applications in food industry for achievement of diverse processing effects. It is among the food additives allowed for application without limitation and according to the applicable legislation (Rozporządzenie Ministra Zdrowia... 2008) the maximum lactic acid dose added to is defined by the liberal term "quantum satis". According to the European Union numeric identification system lactic acid is identified as E 270.

In the literature available there is a vast volume of data on widely understood lactic acid application in, e.g. pharmaceutical, cosmetic, and food industries, in meat processing and little information on lactic acid influence on

Salmonella spp. present in turkey carcasses (MIKOŁAJCZYK, RADKOWSKI 2002a); there is no reports, however, on the influence of lactic acid on *Salmonella* spp. during storage of carcasses.

As a consequence, it seems that undertaking studies aiming at determination of lactic acid influence on *Salmonella* spp. cells present in samples from turkey carcasses stored for the period of 6 days, as that is the usual maximum time before the carcass reaches the consumer, could contribute to improvement in food hygiene level and human health protection.

Materials and Methods

Studies were conducted on 192 samples of turkey breast purchased from poultry plants. Each sample was divided into two parts after weighing and marking. One part was checked for natural presence of *Salmonella* spp. while the other was purposefully contaminated with the test strain.

S. Enteritidis no. 33/66 obtained from the Museum of Bacterial Strains of the National Veterinary Research Institute in Puławy was used for the studies.

The strain was maintained on agar slopes in a refrigerator at 4°C. The above strain was inoculated on nutritive broth and incubated at 37°C for 24 hours. Following incubation, 10 ml of broth was transferred to 4 l of the liquid for dilutions consisting of: peptone 1 g, sodium chloride NaCl 8.5 g, distilled water 1000 ml (Mięso i przetwory mięsne... PN-A-82055-3 1994), in which turkey breast samples were immersed. After 5 minutes the samples were taken out, drained for 2 minutes, placed on specially prepared sterile trays with dripper and held in the refrigerator at 4°C for 20 minutes. For each series of tests the initial contamination level of control samples was determined.

Next the samples were transferred to sterile beakers with 250 ml of 1 and 2% solution of lactic acid (C₃H₆O₃) pure for analyses for 15 minutes.

The controls consisted of breast samples artificially contaminated with *Salmonella* and samples immersed in sterile water for 15 minutes that were tested directly without decontamination (the number assumed as inoculum).

Swabs were collected from the external and internal turkey breast surface using sterile tampons and patterns. The pattern of stainless steel with window area of 25 cm² was placed on each surface tested. Two swabs were collected, one from the external surface and one from the internal surface, representing in total the area of 50 cm². The tampons with swabs were placed in beakers with pearls containing 50 ml of the liquid for dilutions and shaken for ca. 2 minutes. In this way the initial dilution was obtained in which 1 ml of the liquid corresponded to 1 cm² of tested surface. Next 10-times dilution was prepared from that liquid and the number of *Salmonella* spp. colony forming

units was determined by the Most Probable Number (MPN) method (Microbiology of food... ISO 7218:1996). For that purpose from the initial suspension and its consecutive 10-times dilutions 1 ml was inoculated to three parallel tubes containing buffered peptone water (BPW, CM 509, Oxoid Basingstoke Hampshire, UK), that were incubated at 37°C for 20 hours and next transferred on the S.C. medium (SC, 0 687-17-1, Difco Laboratories Detroit MI, USA), on Müller-Kauffman medium (MK, CM 343, Oxoid Basingstoke Hampshire, UK) and Rappaport-Vassiliadis medium (RV, CM 669, Oxoid Basingstoke Hampshire, UK). After 24 hours of incubation at 41.5°C (RV) and 37°C (MK and SF) transfer on brilliantine green agar (BGA, CM 329, Oxoid Basingstoke Hampshire, UK), BSA medium (BSA, 00 73-01-1, Difco Laboratories Detroit MI, USA) and XLD medium (XLD, CM 469 Oxoid Basingstoke Hampshire, UK) was performed. The most probable numbers of *Salmonella* spp. was read from tables by Hoskins.

The tests were conducted according to the methodologies specified in regulations (Mikrobiologia... PN-ISO 6579: 1998, Microbiology... ISO 6579: 1993, Microbiology of food... ISO 7218:1996).

Each turkey breast was tested for determination of *Salmonella* spp. numbers immediately as well as 2, 4 and 6 days of keeping in a refrigerator at 4°C. The tests for each variant were carried out in six repetitions.

The experimental data was processed statistically using the T-Student test and correlation analysis. The correlation analysis was conducted on log numbers.

Results and Discussion

Table 1 and Figure 1 present the changing numbers of *Salmonella* spp. during storage of turkey carcasses at 4°C. The average initial contamination of turkey carcasses' elements with *Salmonella* spp. was $2.4 \cdot 10^3$ of *Salmonella* bacteria. Following immersion in water the average of $4.3 \cdot 10^2$ *Salmonella* spp. cfu were recovered and that number was assumed as the inoculum. The initial inoculum of *Salmonella* enteritidis was 10^2 cfu per 1 cm² of turkey carcass surface. The number of *Salmonella* spp. cfu decreases during storage of turkey carcasses in the refrigerator at 4°C.

As compared to elements immersed in sterile water, the largest decrease, by 2 log cycles, was recorded after 2 days of storage for samples treated with 1% lactic acid (Table 1, Figure 1). In other experimental variants where 1% lactic acid was applied, *S. Enteritidis* grew in numbers that were within the same log range. As compared to samples immersed in sterile water, 2% lactic acid resulted in reduction of *Salmonella* spp. on elements of turkey carcasses by one

Table 1
Influence of lactic acid on survival of *Salmonella* Enteritidis on elements of turkey carcasses stored at 4°C for 2, 4 and 6 days ($n = 6$)

Test substance	Concentration (%)	Days of storage				Correlation between the number of cfu and storage time	
		immediately after contamination	2	4	6	correlation coefficient r	significance level p
		number of cfu					
Control	0	$2.4 \cdot 10^3$	$4.3 \cdot 10^2$	$4.3 \cdot 10^2$	$4.3 \cdot 10^2$	- 0.77	> 0.10
Water	0	$4.3 \cdot 10^2$	$2.4 \cdot 10^2$	$9.3 \cdot 10^1$	$4.3 \cdot 10^1$	- 0.99	0.01
Lactic acid	1	$2.4 \cdot 10^2$	$2.3 \cdot 10^0$	$9.3 \cdot 10^1$	$4.3 \cdot 10^1$	- 0.09	> 0.10
	2	$2.3 \cdot 10^1$	$2.3 \cdot 10^1$	$2.4 \cdot 10^1$	$9.3 \cdot 10^0$	- 0.75	> 0.10

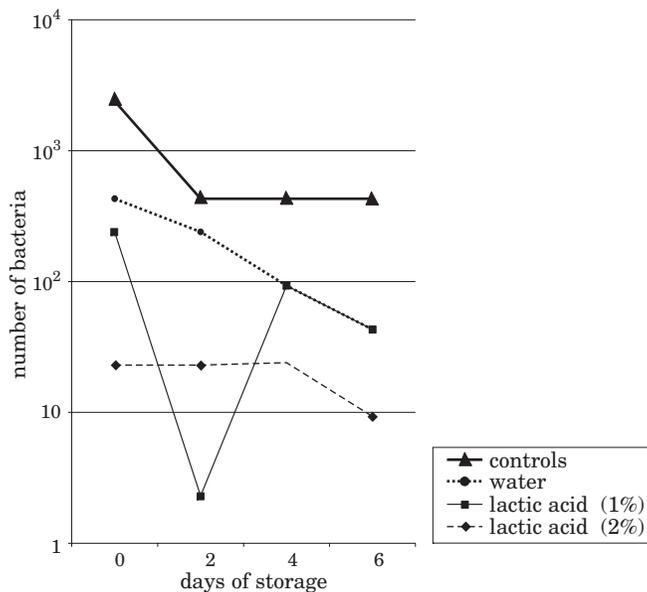


Fig. 1. Influence of lactic acid on survival of *Salmonella* Enteritidis on elements of turkey carcasses stored at 4°C for 2, 4 and 6 days

log cycle immediately after contamination as well as after 2 and 4 days of storage; unfortunately after 4 days of storage *S. Enteritidis* grew in numbers within the same log range.

After slaughter poultry carcasses the temperature within the muscles of which is ca. 40°C, must be chilled down to 4°C to increase shelf life of the meat and inhibit microorganic growth.

If turkey carcasses are contaminated with *Salmonella* spp. during processing, currently applied processes do not contribute to eliminating them. Air temperatures of 0°C to -1°C do not cause elimination of *Salmonella* and carcasses contaminated can be released for sale. The time between chilling and getting the carcass to the consumer varies and depends on numerous factors. The producer defines on the basis of shelf life tests the shelf life of fresh poultry.

The eliminating mechanism of treatment with organic acids in relation to *Salmonella* spp. is related to the presence of dissociated molecules and low pH of the acids (CONNER, BILGILI 1994). The acids applied are dissociated to a different extent and probably that entire combination of hydrogen ions with acid residues creates more unfavourable conditions for *Salmonella* than is the case in case of individual acids.

In own studies the influence of lactic acid solution on *Salmonella* spp. under conditions nearest to the natural, i.e. poultry carcasses coming directly from poultry processing plants not subjected at the laboratory to any processes aimed at liquidation of accompanying microflora and stored under conditions of constant temperature of 4°C was investigated.

BENEDICT et al. (1991) found that high level of bacterial suspension adhesion to skin is achieved through immersion of carcasses in bacterial suspension. The serotype of *Salmonella* spp. and bacterial suspension temperature do not influence adhesion of microorganisms to the skin (CONNER, BILGILI 1994). Cellular structures such as fimbriae and cilia are important in the mechanism of adhesion to the skin (DICKSON 1992, GRAFT-HANSON de, HEATH 1990). The contact time of bacterial suspension with the skin plays a very important role (CONNER, BILGILI 1994). Immediately after inoculation of the studied bacterial culture on the skin the samples should be kept for an appropriate time to obtain better adhesion of the rods to the skin of carcasses. According to CONNER, BAGGILY (1994), 10 minutes is the optimal time required for settlement and adhesion of *Salmonella* spp. to the skin if the inoculum of 10^4 is applied. In case a lower concentration inoculum is applied the holding time should be extended, e.g. for inoculum 10^3 – 20 minutes, for 10^2 – 30 minutes.

Salmonella cells firmly attached to the skin are much more resistant to chemical media than those that did not have the time to attach strongly and that are loosely attached to the skin (LILLARD 1989a, 1989b, TAMBLYN et al. 1997). BAILEY et al. (1986) observed 90–96% reduction of *S. Typhimurium* caused by 3.5 second spray using sodium hypochlorite at 20–40 ppm. Methods of that type frequently decrease the number of, but rarely eliminate *Salmonella* spp. from poultry carcasses as they are ineffective for bacterial cells set or firmly attached to the skin. As a consequence there is need for

testing media eliminating *Salmonella* spp. firmly attached to the skin (TAM-BLYN, CONNER 1997, CONNER, BILGILI 1994).

The critical point in studies on eliminating *Salmonella* spp. is that the bacteria can be firmly attached to the skin, particularly when the carcasses are at the initial stages of processing. It may even happen that *Salmonella* spp. cells are irreversibly attached to the skin. As a consequence it should be considered that no potential medium would be fully effective (CONNER, BILGILI 1994).

Another problem in effectiveness assessment of *Salmonella* spp. eliminating media is the fact that in spite of applying a variety of carcasses washing, washing-out and multiple rinsing techniques attempts at recovering all rods are unsuccessful (IZAT et al. 1991). LILLARD (1989b) concluded that recovery of bacteria attached to poultry skin is highly difficult and secondary rinsing can lead to recovering high numbers of them. In many works (CONNER, BILGILI 1994, IZAT et al. 1991) it is reported that recovery of *Salmonella* spp. is based on multiple washing and even then not all of them can be recovered.

As shown by the literature discussed and own studies, elimination of *Salmonella* spp. from poultry carcass is a complex process that requires further studies.

Conclusions

1. Lactic acid applied for elimination of *Salmonella* spp. from elements of turkey carcasses stored at 4°C for 2, 4 and 6 days showed a relatively strong influence at concentrations of both 1% and 2%, which depended on the time of storage.

2. During storage of test turkey samples at 4°C for 2, 4 and 6 days the number of *Salmonella* spp. cells decreased.

3. The largest decrease in the number of *Salmonella* cells as compared to samples immersed in sterile water, was recorded after 2 days of storage after application of 1% lactic acid.

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