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THE INFLUENCE OF PH AND TEMPERATURE ON *SALMONELLA SPP.* FROM FRESH, CHILLED AND FROZEN POULTRY CARCASSES

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ABSTRACT – The trials were conducted in a slaughtering unit from Iaşi, where 192 samples from poultry carcasses were gathered and analysed microbiologically. Carcasses were washed in order to obtain test samples. The main sources of salmonellosis were poultry and poultry products. *Salmonella* is one of the most important worldwide causes of foodborne diseases. In order to reduce the contamination, we have treated experimentally the washing water with lactic acid sol. 1%. Thus, we stopped the evolution of the microorganisms susceptible to environmental pH changes. This pH change reduced the microbial load, especially of *Salmonella spp,.* from the surface of the carcass. The pH of the carcass washing water was 6.75; after the addition of lactic acid, it reached 2.34 and the temperature of washing water was 15^0C. The contamination of fresh carcasses with *Salmonella spp* had an incidence of 14.06%. After the treatment of washing water, it decreased until 4.6%. In case of 4^0C chilled carcasses, the incidence of *Salmonella* species in the presence of untreated carcasses was of 4.6%, being reduced to 1.5% after treatment.

Key words: decontamination, lactic acid, poultry, *Salmonella spp.,* skin

REZUMAT - Influența pH-ului şi a temperaturii asupra speciei *Salmonella spp.* de pe carnea de pasăre proaspătă, refrigerată şi congelată. Cercetările s-au desfăşurat într-un abator din Iaşi, unde au fost colectate şi analizate din punct de vedere microbiologic 192 de probe de pe carcasele de pasăre. Probele au fost obținute în urma spălării carcaselor. Principalele surse de *Salmonella* le reprezintă pasările şi produsele din carne de pasăre. Cea mai importantă cauză mondială a toxiinfecțiilor alimentare o reprezintă *Salmonella.* Pentru a reduce contaminarea, s-a recurs, experimental, la tratarea apelor (lichidului) de spălare cu soluție de acid lactic 1%. În acest fel, s-a stopat înmulțirea microorganismelor sensibile la schimbările de pH ale mediului. Această schimbare a pH-ului a redus încărcătura microbiologică a probelor, în special cea reprezentată de

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speciile de *Salmonella,* ce proveneau de pe exteriorul carcasei; pH-ul inițial al apei de spălare a carcaselor a fost de 6,75, iar după adăugarea de acid lactic a ajuns la 2,34, temperatura apei de spălare fiind de 15^0C. Contaminarea carcaselor proaspete cu *Salmonella spp.* a avut o incidență de 14,06%, după tratarea apei de spălare reducându-se la 4,6%. În cazul carcaselor refrigerate la 4^0C, incidența prezenței speciei *Salmonella* la carcasele netratate a fost de 4,6%, reducându-se până la 1,5% după tratare.

Cuvinte cheie: acid lactic, decontaminare, pasăre, piele, Salmonella spp.

INTRODUCTION

The biological hazards in food safety comprise pathogenic bacteria, including *E.coli, Salmonella* and *Campylobacter*.

Through manipulation and processing, the poultry meat may be contaminated during the technological processes. The sources of microorganism contamination are numerous, the most important source being the poultry themselves, which may have in their gastro-intestinal tract a rich and diversity microflora (Şindilar, 1989).

Poultry meat is an important source of human infection with *Salmonella spp,. Salmonella enteritidis* and *Salmonella typhimurium,* which are the most commonly reported serovars isolated from poultry, poultry meat products and human cases of salmonellosis (EFSA, 2004a).

The decontamination of poultry carcasses may result in reducing human foodborne infections. The concentration of the lactic acid within the range of 1−2 % is generally accepted. The acid treatment may be done at any stage of the technological process, in the slaughterhouse (Van der Marel, 1988).

The influence of acid decontamination on the appearance (colour) of carcass surface is differently assessed in the literature.

MATERIALS AND METHODS

The trials were initiated during 2007-2008, in a slaughtering unit from Iasi, where 192 samples from poultry carcasses were gathered and analysed microbiologically. The investigated parameters were represented by *Salmonella spp*. Samples were obtained by fresh water washing of frozen and unfrozen carcasses. The poultry carcasses were analysed, by sampling the neck skin under sterile conditions, and then 25 g were weighed for each sample.

The carcass evaluation was done according to the programs of the Sanitary and Veterinary National Authority for Food Safety: Romanian Standard, International Organization for Standardization 6579/2002, which foresees the identification of *Salmonella* spp./25 g from poultry carcasses (only neck skin). According to the standard, *Salmonella* should be absent in 25 g sample.

Some stages of the Hazard Analysis and Critical Control Point are used in the slaughterhouses for testing *Salmonella spp.*

From the selected carcasses, approximately 25 grams of the neck skin have been sampled in order to prevent supplementary contamination of the

carcases. Skin fragments have been drawn with sterile scissors, put in sterile plastic bags (Stomacher - 305/175 mm) and kept on ice, from sampling to the laboratory. Samples were processed for examination in maximum 5 hours since sampling.

For pre-enrichment, we have used buffered peptone water and kept 25 g/ml sample into 225 ml pre-warmed buffered peptone water for 24 h at the temperature of 37^0C.

The secondary selective enrichment is done on two mediums, a highly selective one (Rappaport-Vassiliadis with soy broth) and a less selective one (Muller-Kaufmann tetrathionate broth with Novobiocin that will allow the development of other related species). We took 0.1 ml buffered peptone water into 10 ml Rappaport-Vassiliadis with soy broth for 24 h at 42^0C and 10 ml ml buffered peptone water into 100 ml Muller-Kaufmann tetrathionate broth with Novobiocin for 24 h at 37^0C.

For the selective isolation, two solid mediums will be used, a highly selective *deoxycholate-citrate*-lactose *agar for* 24 h at 37 ^{0}C and a less selective one (Istrati-Meitert), on which the colonies are green. For confirmation, we take five suspected colonies from each plate, which are inseminated in nutrient agar, incubated for 24 h at 37^0C. The confirmation was done on Triple Sugar Iron, Urea Motility Indol and strips API 20E (bioMérieux).

In this study, we have followed the action of lactic acid sol. 1% on microbial strains. The decontamination solution was prepared by diluting 1 % of L (+) lactic acid in water.

The pH measurement was done by using a pH meter. The first pH measurement was done for carcass washing water, indicating a pH of 6.75 and then it decreased until 2.34 in 1% solution of lactic acid.

RESULTS AND DISCUSSION

Although *Salmonella* is recognized as the most important pathogen associated to poultry, it is not known the real incidence of the disease, caused by poultry meat consumption by humans (D'Aoust, 1989). A significant contamination occurs after evisceration. It may appear without a visible carcass contamination with faeces.

Immediately after slaughter, carcasses are immersed in water tanks at 5-15^0 C. In these tanks, carcasses are washed and then are chilled at 4^0C in special rooms. After evisceration, some of the carcasses have a raised microbial load and represent a contamination source.

In many countries, various methods are used to diminish the pathogen load from the carcass surface. The used physical methods are water washing, carcass immersion into hot water and acid solutions.

The use of lactic acid solutions at concentrations of 1–2% diminished (0.8–2.3 log units) the poultry carcass bacterial load, immediately after poultry slaughter and during storage, without affecting the organoleptic characteristics, such as colour and flavour (Smulders, 1995; USDA-FSIS, 1996; Dincer, 2002).

By 1995, Smulders showed that lactic acid was efficient in reducing the microbiological load with 90-99% and pathogens with 30-90%.

The incidence of bacteria from the genus *Salmonella* in/on poultry

varied from one geographical area to another, the reported data alternating between 4 and 100%. The incidence was highly influenced by some factors like growth process and climate conditions. There are countries (Sweden), where poultry are *Salmonella* free. This stage was reached after observing some governmental control programs and measures, applied by poultry breeders and meat processors.

In slaughterhouses where a high number of poultry is processed (thousands poultry/hour) with the same installation, crossed contaminations are more frequent.

The obtained results showed that 14.06% of fresh meat was contaminated with *Salmonella spp.,* 7.8% of chilled carcasses (4^0C) and *Salmonella* free carcasses after freezing (-18^0C) (*Table 1*).

Table 1 – Values of *Salmonella spp*. from fresh, chilled and frozen carcasses

Poultry carcasses	No. samples	Positive samples		Negative samples	
		No.	%	No.	%
Fresh	64	9	14.06%	55	85.9%
Chilled	64	5	7.8%	59	92.1%
Frozen	64	-	-	64	100%

The microorganisms from an acid medium were affected, on the one hand, by free hydrogen ions (H +), that is by pH, and, on the other side, by the concentration of weak acids, which were present in the medium affected by pH.

The anions of some weak acids (e.g., lactic and acetic acids) are metabolized in the microbial cells, releasing H^+ ions, leading to the acidification inside the cell at levels that will stop its metabolic activity, causing death or inhibiting cell multiplication (Carp-Cărare, 2001). The lactic acid is the best-known organic acid in nature, being commonly used in meat processing, its antimicrobial activity being particularly severe at concentrations of 1% in Gram-negative bacteria. The Log reduction has frequently dropped as concerns the sterilizing effect (Snijders, 1985).

In order to reduce the microorganism load from carcass washing water, we have treated it with lactic acid sol. 1%. The pH decrease diminishes the microorganism growth and therefore, *Salmonella spp.* is limited (*Table 2*).

After the primary treatment with lactic acid sol. 1%, carcasses were refrigerated for 24 h. In order to estimate the concomitant bacteriostatic effect of low temperature and low pH medium, samples were gathered after refrigeration, too (*Table 3*).

The combined bacteriostatic effect of both low pH and low temperature proved to be very effective against the monitored pathogen, *Salmonella spp.*

Table 2 – Values of *Salmonella spp* from fresh carcasses after the immersion in lactic acid solution 1%

Species	Samples	Without lactic acid 1%				With lactic acid 1%			
		Positive samples		Negative samples		Positive samples		Negative samples	
		No.	%	No.	%	No.	%	No.	%
Salmonella spp.	64	9	14	55	85.9	3	4.6	61	95.3

Table 3 – Values of *Salmonella spp.* after treatment with lactic acid solution 1% and refrigeration

Species	Samples	With lactic acid 1%				4^0C with lactic acid 1%			
		Positive samples		Negative samples		Positive samples		Negative samples	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
Salmonella spp	64	3	4.6	61	95.3	1	1.5	63	98.4

CONCLUSIONS

The washing tank represents an important source of contamination.

The potential sources of *Salmonella spp*. for humans are poultry meat.

In the poultry sector, *Salmonella* is the most important pathogen of poultry meat.

Organic acids have an important antimicrobial role.

Changing the water pH with a lactic acid solution 1% reduced *Salmonella spp*. growth.

Lactic acid is used frequently in food industry, due to its antimicrobial effect.

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