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The Effect of the Chiller Tank on the Decrease of Indicator Microorganisms on Poultry Carcasses in Two Slaughterhouses in the Rio Grande Do Sul State, Brazil

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Abstract: Indicator microorganisms provide information about the hygienic and sanitary conditions during food processing and storage and may cause deterioration and a reduced shelf life of the product. This study evaluated the reduction of microbial contamination on poultry carcasses after the chiller tank through the enumeration of *Enterobacteriaceae* and total mesophilic aerobic microorganisms. During 2008, 160 samples of poultry carcasses were collected from two poultry slaughterhouses located in Rio Grande do Sul, Brazil. We applied violet red bile glucose agar for the enumeration of *Enterobacteriaceae* and standard agar for the total mesophilic aerobes counting. Slaughterhouse B showed a higher contamination by both *Enterobacteriaceae* and total mesophilic aerobic bacteria than slaughterhouse A. However, statistically, the cooling process showed a significant decrease of microorganisms which evidences the effectiveness of this kind of treatment.

Key words: Chiller tank · Enterobacteriaceae · Total mesophilic aerobic count · Poultry carcasses

INTRODUCTION

Rio Grande do Sul ranks third among poultry exporting states in Brazil, reaching 775,685 tons, right behind the states of Paraná and Santa Catarina. In 2009, until June, exports totaled 329,000 tons [1]. The productive chain of poultry, the cheapest and most accessible protein source [2], depends on the quality and biosafety of the products that are offered to the population.

Poultry meat is highly perishable because its pH is localized near neutrality, it is high in nutrients and there is high water activity. All these factors contribute to the development of microorganisms from the meat itself or from external sources [3]. The intensive processing of poultry products requires constant inquiries about their microbiological quality [2].

The accelerated pace of slaughter and large consumption of poultry have enlarged the problem of bacterial contamination [4]. The cooling step is considered as one of the most important stages of this industrial process, [5] an alternative for the decontamination of carcasses by satisfactorily reducing the number of microbial contaminants [6]. In Brazilian industries, this process is performed by immersing the carcasses in tanks that contain chilled water. Before the chilling process, carcasses have a temperature of 40°C. After immersion, this temperature goes down to below 4°C.

In processed foods, a high number of coliform bacteria or aerobic mesophiles, classed as indicator microorganisms, indicate an inadequate treatment and/or post-processing contamination via unsuitable contact of the product with organic materials and/or dirty equipment or even poor hygienic handling [7].

This study aimed at evaluating the decrease of indicator microorganism contamination of post-chiller poultry carcasses from two slaughterhouses through determination of *Enterobacteriaceae* and total mesophilic aerobic bacterial count.

MATERIALS AND METHODS

In 2008, 160 poultry carcasses samples were taken from two slaughterhouses, 120 samples from slaughterhouse A and 40 samples from slaughterhouse B,

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both located in the state of Rio Grande do Sul. The carcasses were collected before and after the chiller tank, preferably in the morning. The samples were placed in sterile bags and kept in isothermal boxes with ice packs while they were taken to the Food Microbiology Laboratory of the Centro Universitário Univates.

The samples were processed according to the Normative Instruction No. 62 of the Brazilian Ministry of Agriculture, Livestock and Supply [8]. Twenty-five g of skin and muscles of each carcass were removed aseptically and wrapped in sterile polythene bags (Nasco, EUA). Then, 225 ml of 0.1% sterile saline peptone water was added to the bags. These samples were homogenized in a Stomacher (Interscience, EUA) for a minute. The solutions were diluted by adding a 0.1% saline peptone solution. Successive decimal dilutions were prepared from the initial dilution (10^{-1}).

For the *Enterobacteriaceae* count, violet red bile glucose (VRBG) agar (Oxoid, England) was used by incubating the plates at 36°C for 24 hours. Suspected colonies (red, surrounded or not by a zone of precipitated bile acids) were taken into account for count. After performing the oxidase test (Laborclin, Brazil), colonies giving negative reaction were submitted to Gram staining. Three colonies were tested through the Gram staining, the Gram-negative ones were considered belonging to the *Enterobacteriaceae* and the results were expressed as CFU/g.

For viable mesophiles aerobes count, plate count agar (PCA) (Oxoid, England) was used. Following the inoculation, plates were incubated at 36°C for 48 hours. After carrying out the count of the plates, the results were expressed as CFU/g. Begin new paragraph The t-Student's test was applied for the statistical analysis with two paired samples for the averages at a significance level of 5% ($p<0.05$), through the Excel / Windows 2007 program.

RESULTS AND DISCUSSION

The results evidenced that in slaughterhouse A, the mesophilic microorganisms average count was 4.27 log10 CFU/g before the chiller tank and 3.89 log10 CFU/g after it (Figure 1). There was a significant difference between the carcasses contamination before and after the chiller ($p=0.0024$). This fact is probably due to adequate control of water quality (controlled temperature and chiller tank water replacement between shifts) used in this stage.

It was noticed that the process was not effective for some samples evaluated over the year, for both the populations of aerobic mesophilic microorganisms and *Enterobacteriaceae*, as seen in samples 7, 11 and 19 (Figure 1), they were increased instead of decreasing. This fact can be attributed to several factors, such as inadequate quality of water supply and/or inadequate temperature of the water in the chiller tank, as well as prolonged use of the water in the tank [9].

Figure 2 shows a significant decrease ($p=0.0021$) of the enumeration of *Enterobacteriaceae* in slaughterhouse A. Prior to the chiller tank, the average was 2.29 log10 CFU/g, but after it, the average was 1.66 log10 CFU/g. But the process was not effective for sample 8 (Figure 2). Continue with the following paragraph

A study by Northcutt [10] showed that, before the immersion in the chiller tank, the number of total aerobic microorganisms, *Escherichia coli*, *Enterobacteriaceae* and *Campylobacter* was 5.2, 4.5, 3.8 and 4.8 log10 CFU/g, respectively. The immersion in the chiller tank reduced the total aerobic microorganisms, *E. coli*, *Enterobacteriaceae* and *Campylobacter* between 1.2 and 3 log10 CFU/g. In this study, it was possible to observe a similar decrease of mesophiles. The average was 4.27 log10 CFU/g before the chiller tank and 3.89 log10 CFU/g after it. The enumeration of *Enterobacteriaceae* showed an average of 2.29 log10 CFU/g before the tank and 1.66 log10 CFU/g after it.

The Brazilian legislation [9] set a limit of 10^4 CFU/g (equivalent to 4.00 log CFU/g) for refrigerated, frozen or fresh poultry (whole carcasses, portioned or in pieces). The *Enterobacteriaceae* population of slaughterhouse A (Figure 2) lies within the limit established by the Ministry of Agriculture. However, some samples of the same mesophilic microorganisms found in the same place exceed the limits set by the legislation (Figure 1).

Rodrigues [11] analyzed mesophilic aerobic bacteria, total coliforms and *E. coli* in the following stages of processing: before the first washing shower (A), after the first washing shower (B), after manual evisceration (C), after the final washing shower (D) and at the exit of the pre-cooling process (E). The results indicate that there was no significant difference ($p<0.05$) between the averages of mesophilic, total and thermotolerant coliforms among steps A, B, C and D. However, the averages for the microorganisms evaluated in phase E (pre-cooling) were significantly lower indicating reduction of the microbiological contamination significantly.

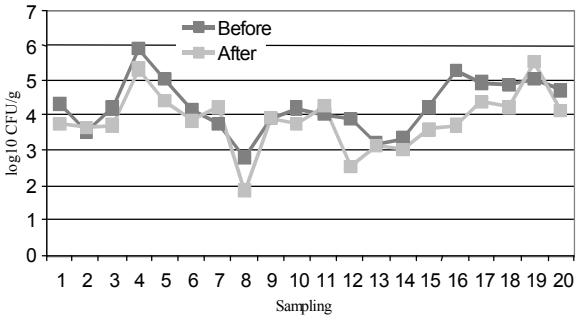

Fig. 1: Heterotrophic microorganisms count (log10 CFU/g) before and after the chiller tank in slaughterhouse A. Sampling 1 to 20 represents 2 different carcasses submitted to analysis in each time (n=120), n=60 before chiller tank and n=60 after chiller tank.

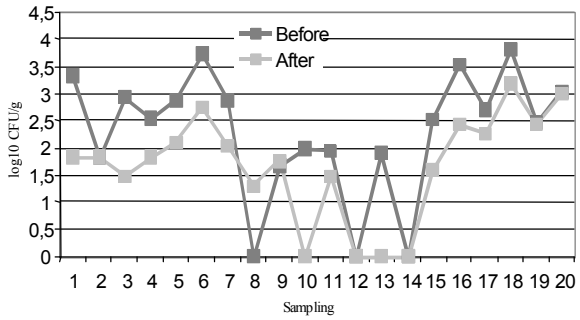

Fig. 2: *Enterobacteriaceae* population (log10 CFU/g) before and after the chiller tank in Slaughterhouse A. Sampling 1 to 20 represents 2 different carcasses submitted to analysis in each time (n=120), n=60 before chiller tank and n=60 after chiller tank.

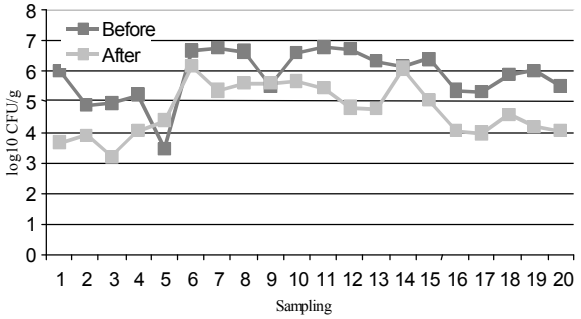

Fig. 3: Heterotrophic microorganisms count (log10 CFU/g) before and after the chiller tank in slaughterhouse B. Sampling 1 to 20 represents 2 different carcasses submitted to analysis in each time (n=40), n=20 before chiller tank and n=20 after chiller tank.

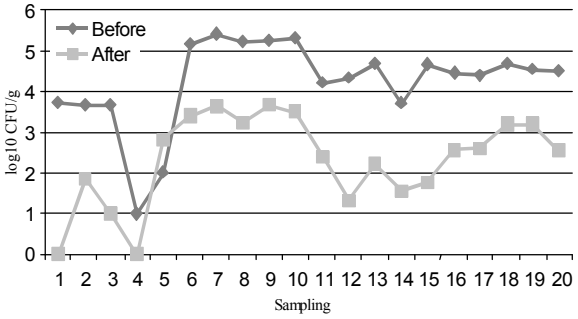

Fig. 4: *Enterobacteriaceae* population (log10 CFU/g) before and after the chiller tank in slaughterhouse B. Sampling 1 to 20 represents 2 different carcasses submitted to analysis in each time (n=40), n=20 before chiller tank and n=20 after chiller tank.

Figure 3 shows a significant decrease (p=0.000123) of the mesophilic population in slaughterhouse B. Prior to the chiller tank, the average was 6 log10 CFU/g. After it, the average was 4.73 log10 CFU/g. A significant similar decrease (p=0.000123) was observed in the populations of *Enterobacteriaceae*, as seen in Figure 4, with an average of 4.22 log10 CFU/g before the chiller tank and 2.31 log10 CFU/g after it.

Most samples of mesophilic microorganisms of slaughterhouse B (Figure 3) exceeded the acceptable levels according to the Brazilian legislation [9], which allows a limit of 10^4 CFU/g microorganisms. A similar phenomenon can be observed in Figure 4; however, only samples collected before the chiller tank showed a high population of *Enterobacteriaceae*.

Mersoni and Garcia [12] found that carcasses collected before the chiller tank had higher levels of *Enterobacteriaceae* contamination than those collected after the tank. The average population of these bacteria before the chiller tank was 5.39 log10 CFU/g, while carcasses showed an average of 2.86 log10 CFU/g after the tank.

Costa and Carvalho [13] developed a plan for hazard analysis critical control points (HACCP) in a production line of whole frozen chicken in a poultry slaughterhouse. First, they determined that the cooling stage of poultry is a critical control point due to the need of slowing the multiplication of bacteria.

Gallardo *et al.* [14] collected poultry samples at three different times representing the beginning, middle and end

of the slaughter morning shift, before the carcasses enter the chiller tank and after they leave it. They observed a significant decrease ($p<0.05$) of total and thermotolerant coliforms and mesophilic aerobes on the carcasses after the transfer from the cooling tanks and at the first collecting time, indicating that pre-cooling tanks were effective in removing microorganisms from the carcasses.

Therefore, the cooling process with water is faster, more efficient and more economically viable [15]. Immersion chillers that use water as a cooling medium, in addition to being faster than the air chillers, prevent carcasses dehydration [16].

According to the Technical Regulation of Technological and Hygienic-Sanitary Inspection [17], water used for renewing the pre-cooling system can be hyperchlorinated, allowing up to 5 parts per million (ppm) of free chlorine. Each tank must be completely emptied, cleaned and disinfected after each work shift (8 hr) or when necessary. Significant reductions of microorganisms may be due to the level of chlorination, adequate equipment cleaning and temperature control of the chiller tank.

Some countries that import Brazilian poultry have prevented commercialization of this product due to residual free chlorine. This chemical is undergoing a number of restrictions due to hazards to human health. Thus, the food industries are continuously seeking alternative processes such as the use of ozone and water supply with a minimum percentage of chlorine to avoid commercial impasses.

The cooling process used in the slaughterhouses that were evaluated was efficient, reducing significantly the mesophilic aerobic and *Enterobacteriaceae* populations, at both slaughterhouses A and B. This control point in poultry slaughterhouses, if properly monitored, ensures that the microbiological quality of poultry meat can even extend the product shelf life.

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