

Effects of hot water surface pasteurisation on lamb carcasses

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Abstract— Hot water pasteurisation of carcasses is accepted as a general intervention in USA, but this is not the case in Europe. The aims of this study were to evaluate the microbiological effects of hot water pasteurisation of lamb carcasses and of recycled hot water used in the pasteurisation cabinet. In addition, the benefits of hot water pasteurisation compared with use of separate meat processing streams for high-risk carcasses in Norway were discussed. Samples (4500 cm²) were collected from 420 naturally contaminated lamb carcasses, half of them subject to hot water pasteurisation at 82 °C for 8 s immediately after slaughter. Hot water pasteurisation significantly reduced the levels of *Escherichia coli*, *Enterobacteriaceae*, *Bacillus cereus* and APC. *E. coli* CFU reduction was 99.5 %. After 24 h storage, the level of *E. coli* was reduced even further, and after five days *E. coli* could not be isolated from the pasteurised carcasses. The recycled water had acceptable physical, chemical and microbiological parameters. Although some carcass discolouration was observed, after 24 h the colour was acceptable. These results suggest that surface pasteurisation could be an important and efficient procedure (critical control point) for reducing generic *E. coli* and thereby shiga toxin-producing *E. coli* on carcasses and thus replace the expensive system of separate processing of high-risk carcass.

Keywords— carcass decontamination, *E. coli*, hot water pasteurisation.

I. INTRODUCTION

There are many decontamination techniques available that can be used to reduce the microbial load on carcasses. Many decontamination studies have been conducted during the past three decades, mainly in USA, and have reported reductions in both spoilage

microorganisms and pathogenic bacteria, by 1-3 log, depending on the initial counts and the decontamination process being investigated [1]. USDA-FSIS has listed decontamination methods for carcasses that can be used without further approval [2]. However, hot water pasteurisation of carcasses has not been accepted as a general intervention in EU. The main concerns are the quality of the recycled water used in the pasteurisation process, and that pasteurisation could mask unhygienic slaughter and dressing [3]. EU regulations permit hot water pasteurisation, depending on water quality and this regulation has been valid in Norway from March 2010. Denmark has been granted an exemption from EU regulations regarding hot water pasteurisation since 2001 for pigs from *Salmonella*-positive herds. The European Food Safety Authority [4] has issued an opinion paper that states that the available data on the efficacy and safety of recycled hot water pasteurisation are very limited, including only treatments of bovine and porcine carcasses, and recommending further research on lamb carcasses. The aims of our study were to evaluate the microbiological effects of hot water pasteurisation of lamb carcasses, both after slaughtering and following subsequent chilling and storage and also to evaluate the recycled hot water. In addition, the benefits of hot water pasteurisation compared with the use of separate meat processing stream for high-risk animals were discussed.

II. MATERIALS AND METHODS

The study was conducted in an authorised abattoir in Norway in October 2009. The slaughter line speed was approximately 250 animals per hour.

Pasteurisation was performed at the end of the slaughter line, after dressing and grading, but before chilling. The hot water pasteurisation system,

consisting of a stainless steel spraying cabinet and a recirculation water system, had been designed and constructed by Banss Schlacht und Födertechnik GmbH (Biedenkopf, Germany) in consultation with the Danish Meat Research Institute at the Danish Technological Institute (Roskilde, Denmark), on commission from Nortura SA (Oslo, Norway). The cabinet (interior dimensions, 0.8 x 2.0 m), had sliding doors at each end, and 16 nozzles on one side and 22 on the opposite side, of which three were dynamic, and were lifted inside the abdomen during spraying. About 130 litres of recycled water and 4 litres of fresh hot water were deluged on each carcass, and fresh, not recycled, hot water was pumped through each of the three dynamic nozzles. The water conductivity was automatically checked at the cabinet, and fresh hot water pumped in when exceeding 300 $\mu\text{S}/\text{cm}$. Stainless filter plates prevented particles from dropping into the recycled water. Temperature and duration of exposure to the pasteurisation conditions were displayed automatically on the exterior cabinet wall. Three carcasses were deluged simultaneously in the cabinet for 8 s.

Sampling

Sampling of freshly slaughtered carcasses was carried out on three consecutive days. In total, 420 lamb carcasses were randomly chosen from more than 30 flocks for inclusion in the study. The sampling numbers and sites were as follow:

- just before chilling, 90 pasteurised, 90 controls
- 24 h after chilling, 90 pasteurised, 90 controls
- five days after chilling, 30 pasteurised, 30 controls

Cotton-cloths (Mesosoft 10x10 cm) were moistened with 10 ml sterile peptone water and rubbed on the outside of the carcasses (approximately 3,000 cm^2), and a second cloth swabbed the inside (approximately 1,500 cm^2 area). The two cloths were placed in the same stomacher bag.

Twelve samples of recycled water were collected from the cabinet in duplicate sets of 500 ml plastic bottles, with or without sodium thiosulphate.

Microbiological analyses

Sterile saline peptone water was added to each stomacher bag and analysed for aerobic plate counts

(APC) by Petri film. *Enterobacteriaceae* was analysed by pour plate method with violet red bile glucose agar according to NMKL method No. 144 with an extra resuscitation start step using the medium Tryptone Soy Agar (TSA). *E. coli* was analysed by pour plate method with violet red bile agar according to NMKL method No. 125 starting with a TSA-step for resuscitation and confirmation by gas production in lactose liquid and indole-positive reactions at 44 °C. *Bacillus cereus* and *Clostridium perfringens* were evaluated by using the Anaerocult system (Merck, Darmstadt, Germany) with *Bacillus cereus* agar (Bc/s, Oxoid) and membrane *Clostridium perfringens* agar (m-CP, Oxoid).

The recycled water samples from the cabinet were analysed for *E. coli* by Colilert Quanti-Tray most probable number method (IDEXX, Westbrook, Maine, USA), heterotrophic plate count (HPC) on blood agar incubated at 22 °C for three days and at 37 °C for two days by ISO Method 6222 (1999), *B. cereus* (Bc/s agar) and *C. perfringens* (m-Cp agar). Turbidity was analysed by ISO Method 7027 (1999), conductivity by ISO Method 7888 (1985), crude protein by Method EEC directive 28 (1993), and fat by Method NS 4752 (1980).

Statistical analyses

The data were transformed from CFU per swab sample (approximately 4,500 cm^2) to $\log_{10}$ for statistical analyses using Stata IC version 11 for Windows. Groups were compared by t-tests and ANOVA, in addition to linear regression analysis. The level of significance was set at $P \leq 0.05$.

III. RESULTS

E. coli

E. coli were isolated from 66 % of control carcasses and from 26 % of pasteurised carcasses at slaughter just before chilling. The average CFU reduction was 99.5 % for pasteurised carcasses (mean value of 77 CFU per carcass) compared with controls (15355 CFU per carcass). The control carcasses had a mean number of 2.39 log CFU per carcass (Fig. 1). The pasteurised carcasses had a mean of 0.54 log CFU per carcass, representing a significant reduction of 1.85 log CFU per carcass ($P < 0.001$). The reduction calculated in log per cm^2 was also 1.85.

For carcasses chilled for 24 h, *E. coli* was isolated from 43 % of the control carcasses and from 21 % of the pasteurised carcasses. The mean value was 0.98 log CFU per carcass for the control group, which was significantly higher than from the pasteurised group, with a mean value of 0.37 log CFU. The mean of *E. coli* on carcasses stored for five days was 0.89 log CFU for control carcasses. *E. coli* was not isolated from the pasteurised carcasses at all.

Linear regression analysis for *E. coli* resulted in the linear equation:

$$Y_{E. coli \log} = 2.06 - 1.18 X_{\text{pasteurisation}} - 0.80 X_{\text{storage1}} - 1.02 X_{\text{storage5}}, \text{ with } R^2 \text{ of } 0.24.$$

Both pasteurisation and storage reduced (negative coefficients) the *E. coli* level on carcasses. Pasteurisation had larger effect (coefficient of -1.18) than storage for 1 or 5 days.

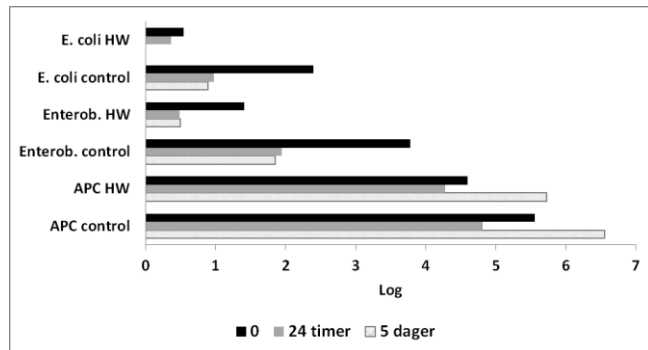


Fig. 1 Mean log₁₀ values per 4,500 cm² from 420 carcass samples by surface swabbing; carcasses subjected to hot water (HW) surface pasteurisation and control carcasses (control). Results from carcasses at slaughter (0 h storage) presented with black bars (n=90 per group), after 24 h storage presented with grey bars (n=90 per group), and five days of storage with white dotted bars (n=30 per group). Results are shown for aerobic plate count (APC), *Enterobacteriaceae* (*Enterobact.*), and *E. coli* (*E. coli*).

Enterobacteriaceae

Enterobacteriaceae were isolated from all the carcasses in the control group, with a mean of 3.78 log CFU per carcass (Fig. 1). *Enterobacteriaceae* were isolated from 66 % of the pasteurised carcasses and the mean was 1.41 log CFU, representing a significant mean reduction of 2.37 log CFU per carcass.

After chilling for 24 h, the mean of *Enterobacteriaceae* was 1.94 log CFU for the control group and 0.49 log CFU for the pasteurised group

($P < 0.001$), while the corresponding numbers after five days of storage were 1.85 log CFU for control carcasses and 0.50 log CFU for pasteurised carcasses.

Linear regression analysis for *Enterobacteriaceae* gave the linear equation:

$$Y_{\text{Enterobacteriaceae log}} = 3.51 - 1.83 X_{\text{pasteurisation}} - 1.38 X_{\text{storage1}} - 1.42 X_{\text{storage5}}, \text{ with } R^2 \text{ of } 0.54. \text{ Both pasteurisation and storage reduced (negative effects) the level of } \textit{Enterobacteriaceae}. \text{ Pasteurisation had the largest effect on } \textit{Enterobacteriaceae} \text{ by a coefficient of } -1.83.$$

Aerobic plate count

The mean value was 5.55 log CFU for controls and 4.60 for pasteurised carcasses freshly slaughtered, representing a significant reduction in APC of 0.96 log CFU. After chilling for 24 h, APC was reduced to a mean of 4.81 log CFU for the control group and 4.28 for pasteurised carcasses. However, after five days the levels of APC had risen to above those for freshly slaughtered carcasses, with a mean of 6.55 log CFU for controls and 5.73 for pasteurised carcasses. Linear regression analysis for APC gave the linear equation:

$$Y_{\text{APC log}} = 5.46 - 0.76 X_{\text{pasteurisation}} - 0.53 X_{\text{storage1}} + 1.06 X_{\text{storage5}}, \text{ with } R^2 \text{ of } 0.50.$$

Pasteurisation and storage for one day reduced the level of APC but storage for five days increased the level by a coefficient of 1.06.

C. perfringens and *B. cereus*

B. cereus was isolated from 23 % (21/90) of the carcasses in the control group with a mean of 0.56 log and from only two pasteurised carcasses with a mean of 0.06 log CFU. The results for *B. cereus* represented a significant 85% CFU reduction due to pasteurisation. Relatively few spore-forming bacteria, *B. cereus* and *C. perfringens*, were detected (Table 1).

Recycled water in the cabinet

E. coli was not detected in the recycled water, and there was also no evidence of multiplication of *C. perfringens* and *B. cereus*. There was no aerobic growth on blood agar at 30 °C, but low numbers of HPC were isolated at both 22 °C (ranging 0-4 CFU/ml) and 37 °C (ranging 0-3 CFU/ml).

The levels of protein ranged between <10 mg/L at the start of the process to 230 mg/L. The fat content

could not be measured in all the water samples due to technical problems. It was also difficult to measure the turbidity and turbidity measurements ranged from 7.4 to 35.9 Formazin Turbidity Units (FTU). Conductivity measurements that were automatically displayed on the cabinet and read while sampling ranged from 76 to 309 $\mu\text{S}/\text{cm}$.

Table 1. Number of carcasses positive for spore-forming bacteria presented as number of positives/carcasses swabbed (%).

Days after pasteurisation	<i>C. perfringens</i>		<i>B. cereus</i>	
	Control, N (%)	Hot water pasteurised, N (%)	Control, N (%)	Hot water pasteurised, N (%)
0	2/90 (2)	1/90 (1)	21/90 (23)	2/90 (2)
1	2/90 (2)	4/90 (4)	2/90 (2)	3/90 (3)
5	1/30 (3)	1/30 (3)	0/30 (0)	0/30 (0)

IV. DISCUSSION

Hot water pasteurisation at 82 °C for 8 s was demonstrated to result in a considerable reduction in the quantity of microorganisms on the surfaces of lamb carcasses. The *E. coli* CFU reduction was 99.5 % and was similar to the results reported by Smith and Graham [5]. Several studies have suggested that hot water pasteurisation (70-96 °C) might be more effective in reducing bacteria than chemicals due to the acid tolerance of the organisms. Sanitizing agents in the water do not always decontaminate more effectively than hot water alone.

Gram-negative bacteria tend to be more heat sensitive than Gram-positive and in our study, the rise in APCs after five days of chilling, might be due to Gram-positive bacteria.

In order to improve slaughter hygiene, the meat industry in Norway has developed a national guideline in which animals representing a higher risk due to faecal contamination (risk animals or risk carcasses), are removed into a separate stream for meat processing. This study indicate that serious consideration should be given to implementing pasteurisation of lamb carcasses prior to chilling as a

replacement for the current expensive system of having a separate stream for processing of risk animals.

Pasteurisation did not eliminate *E. coli*. The water temperature and exposure time could easily be adjusted on the cabinet. Preliminary studies (data not shown) with slightly hotter water and a longer exposure period (85 °C for 12 s), resulted in a greater reduction in quantities of *E. coli* reduction (2.29 log CFU) and less positive pasteurised carcasses (16 %), but resulted in more extensive discolouration of the carcasses. With the temperature and exposure time used in the study, any discolouration was reduced to an acceptable level after 24 h storage in the chiller. The parts of the carcasses that were covered with membranes were less discoloured than tissues with open meat structure and cut surfaces. The extent to which discolouration is acceptable to the consumers has not been investigated.

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