Pre-scald brushing for removal of solids and associated broiler carcass bacterial contamination

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ABSTRACT The aim of the study was to investigate the effect of brushing prior to scalding on reducing the *E. coli* and *Enterobacteriaceae* concentrations on carcasses. Three visits were arranged to a commercial slaughterhouse in which carcasses were cleaned in a separate line. Ten batches were sampled to compare the *E. coli* and *Enterobacteriaceae* concentrations on carcasses before and after a stand-alone brushing unit. Per batch, 8 carcasses before and 8 after brushing were sampled by the whole-carcass rinse method. Furthermore, the dry matter content and the pH were determined in these samples, as these parameters indirectly (dry matter) or directly (pH) influence the scalding lethality. Results revealed a small but statistically significant reduction ($P < 0.001$) in *E. coli* and *Enterobacteriaceae* concentrations on the brushed carcasses. The concentrations on whole carcasses were reduced on average by 0.3 log for both *E. coli* and *Enterobacteriaceae*. Rinse samples from treated carcasses had significantly less dry matter on average by 2.5 g ($P < 0.001$) and significantly higher pH by 0.08 units ($P < 0.001$). Although these differences are statistically significant, they might have rather low biological relevance; thus, further optimization of brushes is needed for more relevant results. This study confirms that brushing reduces bacterial concentrations on carcasses, which may be increased potentially by enlarging the brushed surface of the carcass. Further inline investigations are needed to observe the effect of brushing on bacterial concentrations in scalding water and on carcasses after scalding and at the end of processing.

Key words: poultry, slaughterhouse, scalding, fecal contamination

INTRODUCTION

Fecal contamination is a source of bacteria, e.g. *Escherichia coli* and *Campylobacter*, that contaminate the carcasses during processing. Interventions are needed in order to reduce contamination of carcasses with bacteria; however, the existing measures are either unacceptable for consumers (e.g., chemical decontamination or irradiation [MacRitchie et al., 2014]), or have low effectiveness (e.g., logistic slaughter [Havelaar et al., 2007]). In addition, many of the tested interventions were applied at the end of the processing line (European Food Safety Authority, 2011), where their effect can be inhibited because bacteria become firmly attached to the skin within several seconds (Notermans and Kampelmacher, 1975). This substantiates exploring interventions at the beginning of processing in order to reduce the bacterial concentrations on carcasses entering the processing line and thus on the final product. Little is known about the impact of interventions applied before scalding on bacterial concentrations on carcasses after scalding and at the end of processing. Scalding directly follows bleeding and contributes to significant bacterial reductions via its temperature and washing effects (Berrang and Dickens, 2000; Rosenquist et al., 2006; Pacholewicz et al., 2015; Zweifel et al., 2015). This reduction can be affected by factors such as fecal matter entering the scald tank with broiler carcasses. This material contributes to an increase in the dry matter and a decrease in the pH value of the scalding water (Humphrey, 1981). The dissociation of ammonium urate to uric acid and ammonia results in a decrease in pH (Humphrey, 1981). Maintenance of pH of scalding water at the level of 9 by adding sodium hydroxide was reported to significantly reduce heat resistance of *Campylobacter jejuni* in scalding water and marginally reduce the *Campylobacter jejuni* prevalence

on carcasses after scalding (Humphrey and Lanning, 1987). Also adding acetic acid to scalding water increased the death rate of *Salmonella* Newport, *S.* Typhimurium, and *Campylobacter jejuni* in the water (Okrend et al. 1986). Thus, when the pH in the scalding water is away from the optimum for *Campylobacter* and *Salmonella* heat resistance, the death rate of these bacteria in the water increases. In addition to the pH value, the amount of dry matter, protein content, and temperature of the scalding water were reported to affect the death rate of *S.* Typhimurium in scalding water (Humphrey, 1981; Humphrey et al., 1981).

It is expected that by reducing the fecal contamination on carcasses entering the scalding tank, not only could the bacterial concentration on carcasses be reduced, but also the reduction in pH level of scalding water could be prevented, leading to an increase in bacterial reduction during scalding.

Recently, a prototype intervention was developed by Meyn Food Processing Technology B. V. (Oostzaan, The Netherlands; European Patent Application EP 2 974 601 A1) aiming at mechanical cleaning of broiler chicken carcasses before scalding by a set of brushes. These brushes have bristles shaped as a loop, designed specifically to remove fecal material, especially clumps from the surface of broilers prior to scalding. The goal of this study was to investigate the effect of brushing on bacterial concentrations using *E. coli* and *Enterobacteriaceae* as indicator organisms for fecal contamination. Measuring concentrations of these organisms on carcasses was proposed to indicate the efficiency of control measures in slaughterhouses (European Food Safety Authority, 2012a; European Food Safety Authority, 2012b).

The specific objectives were to 1) investigate whether a reduction in bacterial concentrations through brushing of the carcasses is realized, and 2) to investigate the effect of brushing on the dry matter content and pH.

MATERIALS AND METHODS

Intervention Equipment

A prototype intervention apparatus was designed to brush the breast and vent area of the carcasses before scalding (Figure 1). The purpose of the system was to remove solids such as fecal material, especially fecal clumps, and thus reducing solid matter and bacterial concentrations on carcasses before scalding.

The intervention was a stand-alone unit with 3 brushes that were installed in a stainless steel frame and connected with water and electrical supply. The first brush was designed to brush the vent area of the carcass, whereas the second was aimed at the breast and the third at the neck. The brushes had nylon bristles which were shaped as a loop for better removal of fecal contamination, especially clumps. The length of the bristle of the breast and neck brush was 23 cm and of the vent brushes 27 cm. The bristles had a diameter
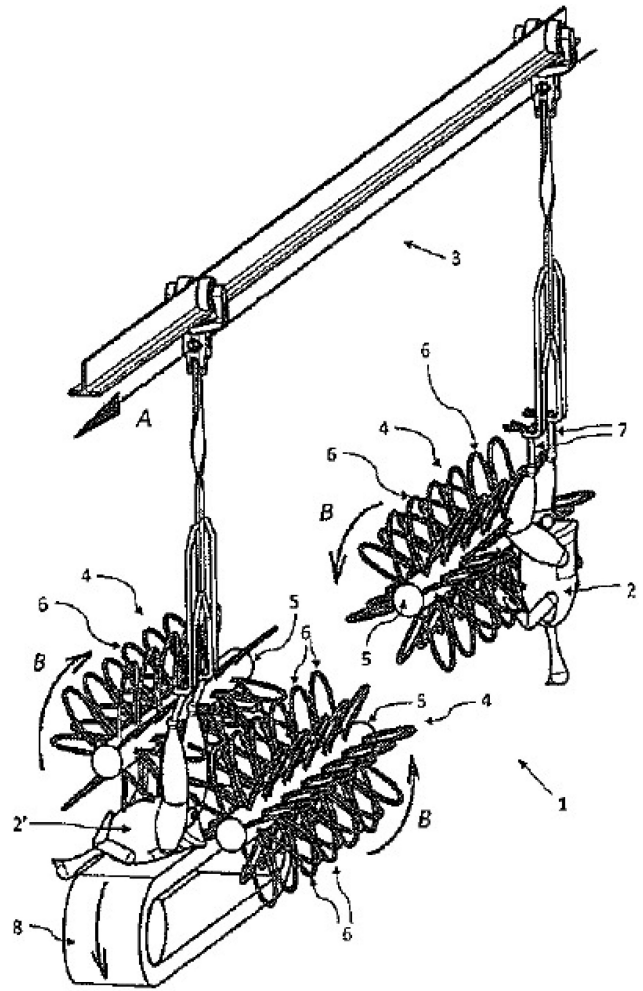

Figure 1. Intervention equipment to brush the exterior of carcasses before scalding. *Source*: European Patent Application EP 2 974 601 A1.

of 1 mm. Bristles were fixed on the shaft of each brush in 12 rows, and each row had 7 bristles, resulting in 84 bristles per brush. The length of each brush was 70 cm and the diameter with bristles was 30 cm for the breast brush and 26 cm for the vent brush. A conveyor belt was present under the vent brush in an effort to tilt and position the carcasses in order to expose the vent area for brushing. The position of the first and second brush in the frame was horizontal, while the third brush was set at an angle in order to brush the neck area of the carcass.

Carcasses were removed from the processing line just after bleeding and placed in the shackles of the brushing unit. First, the carcasses entered the vent brush and were subsequently directed by the shackle guide to the breast brush and then to the neck brush. The vent brush rotated in a downward direction whereas the breast and neck brushes rotated in an upward direction. All brushes rotated with a speed of 2,240 rpm (37 rps). Water sprayers supplied water to clean the brushes. Each brush had five water nozzles providing a flat spraying pattern to enable spraying the whole brushes. The line speed in the brushing unit was 77 carcasses per minute. Water consumption to spray the brushes was 15 L/min,

corresponding with 190 mL/carcass. The water temperature was 16 °C.

Experimental Design

The samples were collected in a Dutch slaughterhouse between October 2014 and February 2015. In total, 10 batches were sampled during 3 sampling visits. Four batches were sampled during the first visit, 3 during the second visit, and 3 during the last visit. In each batch, 16 carcasses were sampled. First, the 8 reference carcasses were collected after bleeding before the brushing. Afterwards, 10 carcasses were removed from the commercial line and hung in the brush unit, treated and 4 carcasses out of the 10 were randomly collected and used to prepare rinse samples. This procedure was repeated once and resulted in sampling of 8 treated carcasses per batch.

The carcasses were sampled by the whole carcass rinse method that was applied according to Pacholewicz et al. (2015) with modification. Prior to rinsing, in addition to cloaca plugging, the head was cut and the neck was closed with a cable tie, and the feet were cut off. Rinse samples were cooled in water with ice and stored at 3°C (±2°C) until laboratory analysis for a maximum of 20 h.

E. coli and Enterobacteriaceae Enumeration

Laboratory analysis was performed on the day following the collection of the samples. The concentration of both *E. coli* and *Enterobacteriaceae* was examined using Petri Films (Petrifilm™ 3 M, Zoeterwoude, the Netherlands, products numbers 6414). Serial dilutions were prepared in Butterfield's Buffer (3 M, Zoeterwoude, the Netherlands, product number BPPFV9BF). After incubation for 24 h at 37 °C, colonies were counted by Petrifilm™ Plate Reader (Model 6499, 3 M, Neuss, Germany). For *E. coli*, blue colonies with gas bubbles were counted, whereas for *Enterobacteriaceae*, red colonies with yellow zones and red colonies with gas bubbles with or without yellow zones were counted. The detection limit was 10 CFU/mL for *E. coli* and 100 CFU/mL for *Enterobacteriaceae*. Results from the whole carcass rinse samples were converted to log CFU/carcass by scaling to the volume of the original rinse sample.

pH and Dry Matter Content

The pH and dry matter content were measured in rinse samples. The pH was measured by pH-Tester (HI 98103, HANNA instruments Deutschland GmbH, Kehl am Rhein, Germany) on the day following collection of the samples. Dry matter was measured by freeze drying of 20 mL of the rinse sample collected in a 50 mL plastic tube. The samples were frozen at –80 °C overnight. Afterwards, the samples were dried for 48 to 72 h in the freeze dry system Freezone 2.5 (Labconco, Beun De Ronde, Abcoude, the Netherlands). After drying, the samples were weighed on a XS105 DualRange scale (Mettler Toledo, Tiel, the Netherlands). The measurement precision was 0.1 mg. Results were expressed as the dry matter content in grams per carcass.

Statistical Analysis

The log normal distribution of *E. coli* and *Enterobacteriaceae* concentrations was checked by diagnostic qq plots.

Data analysis was performed in the R statistical software (3.2.0, 2015, R development Core Team). The effect of brushing in the first experiment on *E. coli*, *Enterobacteriaceae*, dry matter, and pH was analyzed by a linear mixed effect models (Pacholewicz et al., 2015). The models were used to analyze whether the effect of brushing on bacterial concentrations, pH and dry matter was regular or variable between the tested batches. The first model (1), as described below, was used to analyze the data on dry matter content and pH. In the model "Value" is the mean pH or dry matter in the samples collected from reference or treated carcasses. Term "group" indicating reference or treated carcasses, was an explanatory factor and batch was modeled as a random effect of the intercept. The intercept $(b_0 + \beta_0)$ varied between batches, and the effect of brushing (slope) was either regular for each batch (β_1 is a fixed effect, $b_1 = 0$) or varied between batches ($b_1 > 0$, random effect). The second model (2) was used to analyze data on *E. coli* and *Enterobacteriaceae* concentrations. In the model "Value" is the mean of *E. coli* concentrations or *Enterobacteriaceae* concentrations collected from reference or treated carcasses. The intercept and slope are as in model one. In addition, the second model has terms "organism" and interaction of "organism and group", in order to analyze whether the effect of brushing was the same for both *E. coli* and *Enterobacteriaceae*. Significance of the interaction term estimate (β_3) indicates differences in the effect of brushing for both organisms. In both models the residual error (ε) varied over carcasses. The models used are given by,
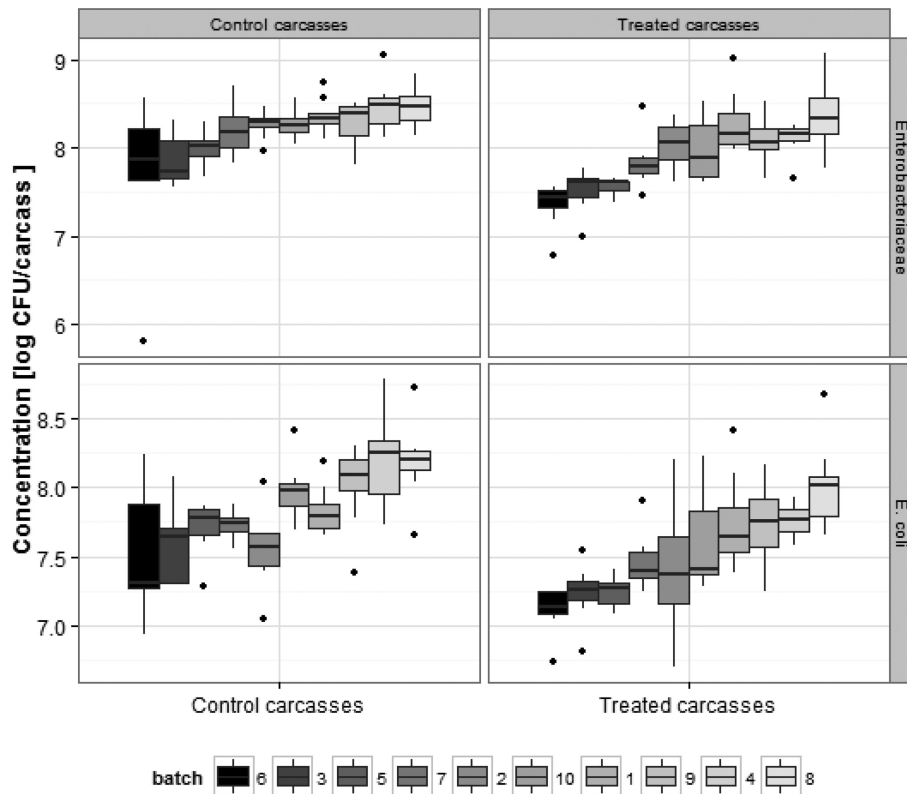
$$Value = b_0 + \beta_0 + (\beta_1 + b_1)group + \varepsilon, \quad (1)$$

$$Value = b_0 + \beta_0 + (\beta_1 + b_1)group + \beta_2\,organism$$
$$+ \beta_3\,group * organism + \varepsilon. \quad (2)$$

RESULTS

Reduction of E. coli and Enterobacteriaceae on Brushed Carcasses

Concentrations of both *E. coli* and *Enterobacteriaceae* in the whole carcass rinse samples before and

Figure 2. Concentrations of *E. coli* and *Enterobacteriaceae* (log CFU/carcass) in whole-carcass rinse samples obtained from reference and treated carcasses in 10 batches (sorted by increasing median). The length of the boxes indicates the interquartile range of concentrations; the whiskers indicate maximum and minimum concentrations; the dots indicate the outliers; and the lines indicate the median.

after brushing are presented in Figure 2. According to the model, the bacterial concentrations on carcasses before brushing varied significantly between batches as indicated by the random intercept in the model. Furthermore, the model indicated that the effect of brushing was not statistically different between tested batches, because the slope was fixed ($b_1 = 0$). The effect of interaction of group and organism shown by estimate (β_3) was not significant ($P = 0.84$), which confirmed that the effect of brushing did not differ between the *E. coli* and *Enterobacteriaceae*. The concentrations on brushed carcasses were reduced on average by 0.3 log ($P < 0.001$) for both *E. coli* and *Enterobacteriaceae* in each tested batch.

Reduction of Dry Matter

Figure 3 represents the weight of dry matter recovered from whole-carcass rinses obtained from the reference and treated carcasses. The model with random intercept and random slope ($b_1 > 0$) fitted the data, indicating differences in dry matter between batches before brushing and in removal of fecal material between batches. According to the model, the average content of dry matter in the rinse samples obtained from carcasses before brushing was 4.5 g (standard deviation 1.6 g), which decreased significantly ($P < 0.001$) after brushing by 2.5 g (standard deviation 1.3 g).

pH

Figure 4 represents the results on pH of the rinse samples collected from reference and treated carcasses by brushes. Here, the model with fixed slope ($b_1 = 0$) and random intercept fit the data best. The results from the model confirmed that the pH varied between batches. According to the model, the average pH value in the rinse samples obtained from the carcasses before brushing was 6.19; in the samples obtained after brushing, the pH value was significantly ($P < 0.001$) higher, by 0.08 on average. This result confirmed that brushing prevented reduction of, and even increased, the pH value in the rinse sample.

DISCUSSION

Slaughterhouses have limited influence on the fecal contamination and thus bacterial concentrations on broilers entering the slaughterhouse, but interventions are needed to reduce bacterial concentrations as early in processing as possible. An intervention to brush the exterior of the carcasses before scalding was tested. A significant reduction of both *E. coli* and *Enterobacteriaceae* concentrations on the treated carcasses by 0.3 log was found. Despite the statistical significance found, based on the obtained results, the biological effect of brushing needs further investigation. This study was done based on the prototype equipment and areas of
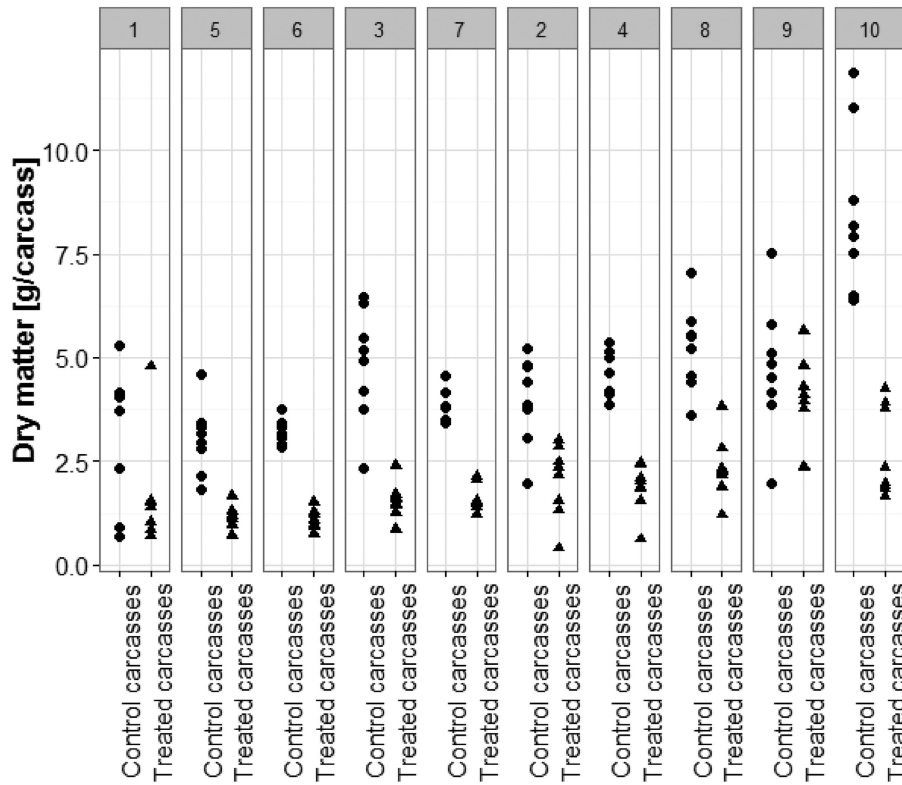
Figure 3. Effect of brushing on the dry matter content in the whole-carcass rinse samples of reference and treated carcasses. The dots present the weight (g/carcass) of dry matter recovered from whole-carcass rinse samples from reference carcasses, whereas triangles from treated in particular batches (1–10). The dry matter content is sorted on the figure by increasing median in the tested batches.
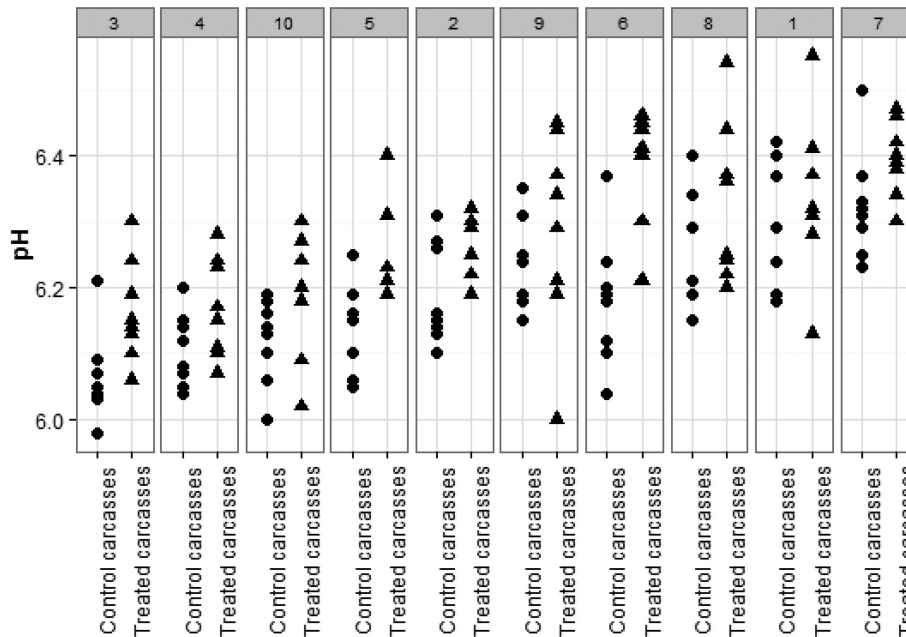
Figure 4. Effect of brushing on change of pH level in the whole-carcass rinse samples collected before and after brushing. The dots present pH in each of the whole-carcass rinse sample from reference carcasses, whereas triangles from treated in particular batches (1–10). The pH value is sorted on the figure by increasing median in the tested batches.

improvements and potential solutions to increase the reduction effect were suggested. Reduction in bacterial concentrations caused by prescald brushes was also evaluated by other authors (Berrang and Bailey, 2009). Although these authors brushed the whole surface of

the carcass, in contrast to selected areas in our study, they observed an insignificant reduction in *Campylobacter* concentrations by 0.46 log and in *E. coli* concentrations by 0.19 log. The differences in reduction observed in our study and by Berrang and Bailey (2009) may

be related to different types of brushes (loop brush in our study versus conventional brush, confirmed by communication with the authors). Data on other parameters applied in that study such as water consumption is not available, making it difficult to further compare the results with our findings. Although the prescald conventional brush tested by Berrang and Bailey (2009) caused insignificant reductions in bacterial concentrations, according to the authors, its combination with other interventions contributed to significant reductions in concentrations at the end of processing. These findings suggest that application of brushes is a potentially effective hurdle to reduce bacterial concentrations on carcasses. The impact of brushes tested in our study on bacterial reductions is expected to increase by brushing a larger area of the carcass. Parts of carcasses other than breast and vent carry bacteria, as demonstrated by other authors (Berrang et al. 2000; Kotula and Pandya, 1995). Therefore, increasing the brushed surface could increase the bacteria reductions.

The brushes tested in our study removed the fecal clumps effectively, in contrast to brushes tested by Shackelford et al. (1992). In that study, the effectiveness of brushes on removal of solids from carcasses before scalding depended on the consistency of the fecal material adhering to the feathers. The differences in the effect of clump removal in our study, compared to the study by Shackelford et al. (1992), may also be explained by the type of brushes used. Comparing the results from Shackelford et al. (1992) and ours, it seems that the loop brush may be more effective in removing fecal material adhering to feathers than brushes with straight bristles. Moreover, in our study, the carcasses were tilted during vent brushing, thereby exposing the vent area and facilitating brushing. Other brushing parameters applied by Shackelford et al. (1992) differed slightly from those used in our study, e.g., 140 mL water used to spray the brush, compared to 190 mL in our study.

Brushing significantly reduced the dry matter content in rinse samples from treated carcasses. Reduction of dry matter differed between the tested batches, in agreement with Shackelford et al. (1992), who reported variations between batches in the total solids removed from carcasses. These authors, despite limitations on effective removal of clumps, reported reduction of solids in the scalding water as a result of application of brushes.

Our results have shown that the pH in rinse samples from treated carcasses was higher compared to reference samples. The difference in pH was relatively small (0.08 units) and the biological relevance needs further investigation, but this result confirms that brushing of the carcasses contribute not only to a decrease in solids, but also reduces the decrease in pH of scalding water. This will potentially increase the lethality of this processing step when inline brushes are applied. As reported previously (Okrend et al. 1986; Humphrey and Lanning, 1987) the pH in the scalding water influences the D_T

of *Campylobacter* and *Salmonella* when the pH is at a value that differs from the optimum for heat resistance for these bacteria. The effect of brushing on bacterial concentrations in scalding water and on carcasses after scalding will be evaluated in further studies.

In summary, the tested brushes are a potentially effective approach to reduce solids and bacterial concentrations on carcasses before scalding. It is likely that the reduction can be increased by brushing larger surface areas of carcasses than tested in this study. Further studies should investigate the impact of brushing on bacterial concentrations in scalding water and on carcasses after scalding and at the end of processing.

ACKNOWLEDGMENTS

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