Journal of Food Protection, Vol. 84, No. 4, 2021, Pages 572–578
https://doi.org/10.4315/JFP-20-395
Published 2021 by the International Association for Food Protection

Research Note

Reducing *Campylobacter jejuni, Enterobacteriaceae, Escherichia coli,* and Total Aerobic Bacteria on Broiler Carcasses Using Combined Ultrasound and Steam

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MS 20-395: Received 30 September 2020/Accepted 9 November 2020/Published Online 12 November 2020

ABSTRACT

Campylobacteriosis is the most frequently reported foodborne illness in Europe and many other parts of the world. *Campylobacter* can colonize the intestines of broilers, mostly in large amounts. Broilers are usually slaughtered in a high-speed automated system that could cause rupture of the intestines during evisceration, resulting in contamination of carcasses with intestinal bacteria like *Campylobacter*. This study evaluated the combined effects of ultrasound and steam (SonoSteam) on naturally contaminated chicken carcasses at a large-scale abattoir in Sweden. Ultrasound at 30 to 40 kHz and steam at 84 to 85°C or 87 to 88°C were used at slaughter, with a line speed of 18,000 birds per hour. The amounts of *Campylobacter* spp., *Enterobacteriaceae, Escherichia coli,* and total aerobic bacteria on neck skins from 103 chicken carcasses, sampled before and after treatment by ultrasound-steam, were analyzed. *Campylobacter* spp. were quantified in 58 (56%) of the neck skins, from birds belonging to four of the seven flocks represented. All 58 isolates were identified as *Campylobacter jejuni*. After the ultrasound-steam treatment, the mean reductions in *C. jejuni, Enterobacteriaceae, E. coli* and total aerobic bacteria were 0.5 ± 0.8, 0.6 ± 0.6, 0.5 ± 0.6, and 0.4 ± 0.7 log CFU/g, respectively. No significant differences in reduction between the two different treatment temperatures were observed for any of the bacteria. Although the bacterial reductions were significant, large amounts of bacteria remained on the carcasses after treatment. Further studies are needed to identify optimal measures at slaughter to reduce food spoilage bacteria and pathogenic bacteria, which should be considered in a One Health perspective.

HIGHLIGHTS

- Ultrasound-steam treatment reduced the numbers of bacteria on broiler neck skin.
- *Campylobacter* was present at >3 log CFU/g on some treated chicken neck skins.
- Reductions in bacterial levels were greatest on carcasses with high initial amounts.
- Higher steam temperature did not result in higher bacterial reductions.

Key words: Broiler carcass; *Campylobacter*; *Enterobacteriaceae*; *Escherichia coli*; Total aerobic bacteria; Ultrasound-steam

Campylobacteriosis has been the most frequently reported foodborne illness in the European Union since 2005 *(6)*. During 2016 to 2018, almost 250,000 confirmed cases were reported each year in Europe *(6),* although the actual number of cases is estimated to be around 9 million per year *(7)*. The total costs related to campylobacteriosis in the European Union are estimated to be around 2.4 billion euros per year *(7)*. *Campylobacter* is highly prevalent in broiler flocks worldwide, and handling and consumption of chicken and contaminated food present a high risk of campylobacteriosis in humans *(3, 13)*. In a baseline study performed in Europe in 2008, *Campylobacter* prevalence in cecum samples was detected in 71% of flocks on average, but it varied considerably (range, 2 to 100%) between different countries *(5)*.

Campylobacter can colonize the intestine of broilers, often in very high amounts (up to 8 log CFU/g), without the birds showing any symptoms of illness *(11, 26, 27)*. The prevalence of contaminated carcasses postchill has been shown to increase with higher degrees of intestinal colonization in the slaughter group *(16)*. High concentrations of *Campylobacter* on chicken meat are associated with an increased risk of disease in consumers *(17, 21)*. Risk assessment studies have shown that the risk of consumers developing campylobacteriosis would be reduced by 50% at the European Union level if all broiler flocks met the

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microbiological criterion of <3.0 log CFU *Campylobacter* per gram on neck skin *(28)*.

In large-scale broiler processing plants, the slaughter process is highly automated and occurs at very high speed. This can cause rupture of the intestines during evisceration, which can result in contamination of carcasses with intestinal bacteria like *Campylobacter*. The numbers of *Campylobacter* on carcasses from slaughter groups with high levels of intestinal colonization have been shown to increase 10-fold compared with those on *Campylobacter*-positive carcasses from slaughter groups with no positive cloacal samples *(16)*.

Different interventions can be performed to reduce the numbers of *Campylobacter* on broilers after slaughter. The efficiency of steam as a disinfection method has been evaluated in several studies *(1, 15, 18, 29)*. Steam pasteurization has been successfully used in a beef processing plant *(25)* and has also been tested on broiler carcasses *(15, 18, 29)*. In some of those tests, the treatment gave significant reductions (up to 3.3 log CFU/cm^2) in the numbers of *Campylobacter* on carcasses but also impaired carcass quality *(15, 29)*. Thus, there is still no solution to this quality problem, and further research is needed *(12)*. To reduce the treatment time, a technology that combines steam with ultrasound has been developed. The ultrasound destroys the protective sublayer of air that is present around all objects, facilitating rapid heat transfer. The steam can then immediately reach the carcass skin. This technology has been evaluated in a few studies and has been found to reduce the numbers of *Campylobacter* on broiler carcasses by 2.5 log CFU/mL *(9),* 1.0 log CFU/g *(20),* and 2.5 log CFU per carcass *(2)*.

The aim of the present study was to evaluate the effects of full-scale ultrasound-steam treatment on broiler carcasses naturally contaminated with bacteria in a slaughterhouse in Sweden.

MATERIALS AND METHODS

Broiler flocks. In order to select appropriate broiler flocks, flocks from producers with a previous history of often delivering *Campylobacter*-positive flocks at slaughter and enrolled in the Swedish Campylobacter program were selected *(10)*. Some of the flocks that were sent to slaughter had previously been tested (using sock samples in the broiler house) for the presence of *Campylobacter* 1 to 2 weeks before slaughter, as part of another study *(8)*. A total of seven flocks from six farms were included in the present study, and 10 to 25 carcasses from each flock were sampled.

Ultrasound-steam treatment. The SonoSteam equipment (European patent EPO; 116 02 722 020.12113, FORCE Technology/Sanovo Technology Group A/S, Brøndby, Denmark) uses a combination of ultrasound at 30 to 40 kHz (25 to 30 kHz) and steam. In this study, steam temperatures of 84 to 85°C and 87 to 88°C were used. The ultrasound-steam chamber was installed at the end of the slaughter line (Fig. 1) in a large-scale broiler chicken processing plant in Sweden. The chamber was positioned to allow whole carcasses on processing-line shackles to be treated before chilling. The ultrasound waves were produced simultaneously with the steam. The chamber contained two rows of nozzles, supplying steam for inside and outside treatment of the carcasses. The carcasses were treated for 1.2 to 1.5 s. After the treatment, the carcasses were sprayed with water and chilled with forced air for approximately 2.5 h.

Sample collection. Carcasses were randomly selected within the flocks selected for the study, but only carcasses without visible contamination and with a sufficient amount of neck skin were sampled. The line speed at the slaughterhouse was around 18,000 birds per hour. The carcasses were removed from the slaughter line and sampled before they entered the ultrasound-steam chamber. For this, approximately 10 g of neck skin was cut aseptically from the left or right side of alternate carcasses and weighed. The carcasses were then each labeled with a red band and placed back on the slaughter line. Each neck skin sample was placed in a separate stomacher bag (Blender bags Standard 400, Grade Products, Coalville, England) without transport medium and was labeled with an individual number and the letter A. The labeled carcasses were sampled in a similar way after treatment, and the bags were marked with the individual number of the carcass and the letter B. In this way, each carcass acted as its own control. Owing to the high line speed and short distance between the exit of the ultrasound-steam chamber and the entrance to the chilling room, the second sampling had to be performed after the chilling area and not immediately after ultrasound-steam treatment. In total, neck skins from 103 individual carcasses were sampled before and after treatment.

The samples were transported on the day of sampling, in an insulated box with refrigerant gel packs, to the laboratory at the Swedish University of Agricultural Sciences. The temperature was checked upon arrival. Only samples with a temperature of 2 to 8°C were accepted for analysis.

Bacteriological analysis. Samples were kept at a temperature of 2 to 8°C at the laboratory until analysis, which began within a maximum of 48 h after sampling at the processing plant. From each sample, 10 g of neck skin was aseptically weighed and placed in a stomacher bag, together with 90 g of buffered peptone water (CM0509, Oxoid, Basingstoke, UK), and homogenized for 1 min at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, NJ). A 10-fold serial dilution of the fluid in peptone water (salt from VWR, peptone from Oxoid) was then prepared.

Enumeration and identification of thermotolerant *Campylobacter* spp. Quantification of thermotolerant *Campylobacter* spp. was determined according to ISO 10272-2 *(14)*. Modified charcoal cefoperazone desoxycholate agars (mCCDA; CM0739, Oxoid) were preincubated at 41.5 ± 0.5°C for 30 min before use. From the initial dilution, 1.0 mL was surface plated equally on four mCCDA plates (9 cm in diameter). For further dilutions, 0.1 mL was surface plated on a single mCCDA plate. All plates were incubated at 41.5 ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere, using gas jars containing CampyGen sachets (Oxoid). A blood agar plate (SVA, Uppsala, Sweden) with *Campylobacter jejuni* (CCUG 43594) was placed in each jar, for qualitative control of the microaerobic atmosphere. After incubation for 44 ± 4 h, colonies characteristic of *Campylobacter* were counted. Bacterial counts were performed on plates with less than 150 colonies, and the number of *Campylobacter* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

When *Campylobacter* was detected, at least three typical colonies from each agar plate were recultured on blood agar and incubated at 41.5 ± 1°C in a microaerobic atmosphere for 48 ± 4

FIGURE 1. *Ultrasound-steam chamber installed in the slaughter line of a large-scale broiler chicken processing plant.*

h. The colonies were then identified to species level using matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Billerica, MA).

Enumeration of bacteria belonging to the family *Enterobacteriaceae*. Enumeration of bacteria belonging to the family *Enterobacteriaceae* was performed according to NMKL 144, 3rd ed. *(22),* using the 10-fold serial dilution described above. A 1.0-mL sample from each dilution was mixed carefully with 10 to 15 mL of violet red bile glucose agar (BD, Sparks, MD) in a petri dish (9 cm in diameter) and left to solidify, and then an overlay of around 5 mL of violet red bile glucose agar was added. The plates were incubated at 37 ± 1°C for 24 ± 2 h. Bacterial counts were performed on plates with 15 to 150 colonies. Five colonies preliminarily identified as *Enterobacteriaceae* were cultured on blood agar and incubated at 37 ± 1°C for 24 ± 2 h. The identity of bacteria belonging to the family *Enterobacteriaceae* was confirmed by oxidase test, and the number of *Enterobacteriaceae* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

Enumeration and identification of *E. coli*. Enumeration of *Escherichia coli* was performed according to NMKL 125, 4th ed. *(23)*. In brief, a 1.0-mL aliquot of each dilution in the initial dilution series prepared for each sample was mixed carefully with 5 mL of tryptic soy agar (TSA; Oxoid) in a petri dish (9 cm in diameter) and preincubated at room temperature (20 to 25°C) for 1 to 2 h. An overlay of 10 mL violet red bile agar (Oxoid) was then added. After solidification, the plates were incubated at 44°C for 24 ± 2 h. Bacterial counts were performed on plates with 10 to 100 colonies. Five colonies preliminarily identified as *E. coli* were cultured on TSA agar and incubated at 37°C for 24 ± 2 h. The colonies were identified to species level using matrix-assisted laser desorption ionization–time of flight mass spectrometry. The number of *E. coli* bacteria was expressed as log CFU per gram, with a detection limit of 1.0 log CFU/g.

Enumeration of total aerobic bacteria. Enumeration of total aerobic bacteria was performed according to NMKL 86, 5th ed. *(24),* on the same 10-fold serial dilution described above. A 1.0-mL aliquot from each dilution was mixed carefully with 15 to 20 mL of plate count agar (Oxoid) in a petri dish (9 cm in diameter) and left to solidify. After agar solidification, the plates were incubated at 30 ± 1°C for 72 ± 6 h. Bacterial counts were performed on plates with 25 to 250 colonies, and the total aerobic bacteria content was expressed as log CFU per gram, with a detection limit of 3.0 log CFU/g.

Statistical analysis. The results were compiled and analyzed using Microsoft Office Excel and R Studio (RStudio version 1.2.1335, Windows 7+). Bacterial counts (CFU per gram) were log transformed. Standard deviations of bacterial reductions following treatments were calculated. Statistical significance was determined by the paired *t* test, which was performed for each of the four bacterial groups studied. The Welch two-sample *t* test was conducted to determine significant differences between the two treatment temperatures (84 to 85°C and 87 to 88°C) for each bacterial group. Analysis with simple linear regression was performed to determine whether the initial amounts of *Campylobacter, Enterobacteriaceae, E. coli,* and total aerobic bacteria on the neck skin influenced the level of reduction achieved by ultrasound-steam treatment. Differ-

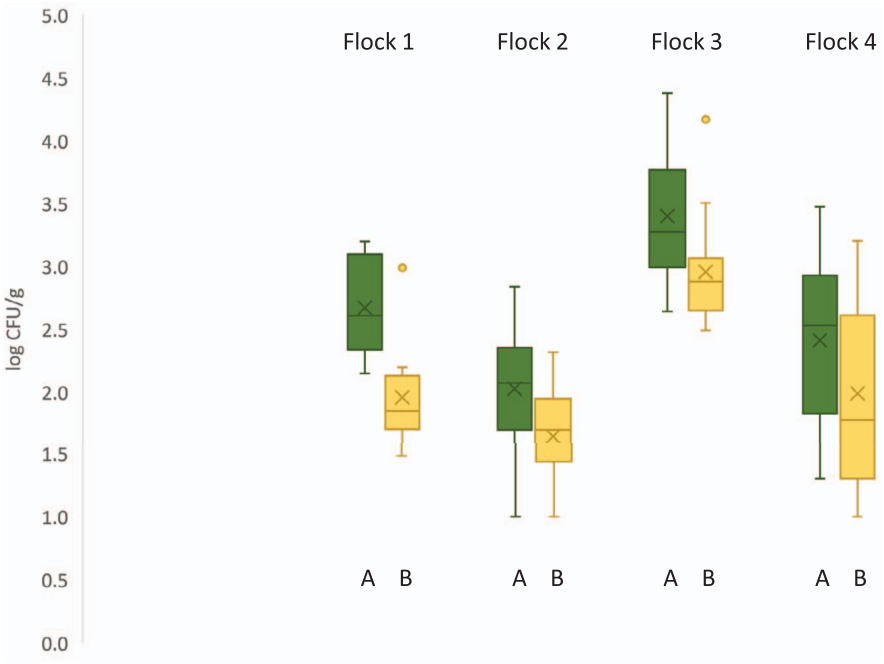

FIGURE 2. *Numbers of* Campylobacter jejuni *bacteria on neck skins from broilers in each of the four flocks that gave positive samples before (A) and after (B) ultrasound-steam treatment.*

ences before and after treatment and between treatment temperatures were deemed significant at a P value of <0.05.

RESULTS

Enumeration and identification of thermotolerant *Campylobacter* spp. *Campylobacter* was detected on 58 (56%) of the 103 neck skins tested, originating from four of the seven flocks sampled. All 58 isolates were identified as *C. jejuni*. One neck skin sample was found to be contaminated with *Campylobacter* at 1.7 log CFU/g before treatment, but no *Campylobacter* was quantified after the treatment. Therefore, this sample was removed from the statistical analysis.

The amounts of *Campylobacter* before treatment by ultrasound-steam varied between 1.0 and 4.4 log CFU/g and after treatment between 1.0 and 4.2 log CFU/g (Fig. 2). The mean reduction in *C. jejuni* was 0.5 ± 0.8 log CFU/g. A reduction in *C. jejuni* after the ultrasound-steam treatment was observed in 46 of the 58 samples, an increase was observed in 7, and 4 of the samples did not show either any increase or reduction. Samples with *Campylobacter* levels above 3 log CFU/g consisted of 14 (24%) of the positive samples before the ultrasound-steam treatment and 7 (12%) after the treatment (Fig. 2). The difference in numbers of *C. jejuni* before and after the treatment was highly significant ($P < 0.0001$).

Enumeration of bacteria belonging to the family *Enterobacteriaceae*. The amounts of bacteria belonging to the family *Enterobacteriaceae* before treatment by ultrasound-steam varied between 1.6 and 5.2 log CFU/g, while after treatment, the amounts varied between 1.1 and 3.7 log CFU/g (Fig. 3). In one pretreatment sample, the amount of *Enterobacteriaceae* was not countable, and therefore, that sample was removed from the analysis. The mean reduction in *Enterobacteriaceae* was 0.6 ± 0.6 log CFU/g. A reduction in the number of *Enterobacteriaceae* after ultrasound-steam treatment was observed for 83 of the 100 samples, while an increase was observed for 16 of the samples. In 17% of the samples, the number of *Enterobacteriaceae* was above 3 log CFU/g after treatment. The difference in numbers of bacteria belonging to the *Enterobacteriaceae* before and after treatment was highly significant ($P < 0.0001$).

Enumeration of *E. coli*. The amounts of *E. coli* before treatment by ultrasound-steam varied between 1.7 and 5.0 log CFU/g, and after treatment, the amounts varied between 1.7 and 4.2 log CFU/g (Fig. 3). The mean reduction in *E. coli* was 0.5 ± 0.6 log CFU/g. A reduction in *E. coli* after the ultrasound-steam treatment was observed in 80% of the samples, while an increase was observed in 20% of the samples. After the treatment, the number of *E. coli* was above 3 log CFU/g in 36% of the samples and above 4 log CFU/g in 3% of the samples. *E. coli* was isolated from all 100 samples, both before and after treatment by ultrasound-steam. The difference in numbers of *E. coli* before and after treatment was highly significant ($P < 0.0001$).

Enumeration of total aerobic bacteria. The amounts of total aerobic bacteria before treatment by ultrasound-steam varied between 3.7 and 7.0 log CFU/g, and after treatment, the amounts varied between 3.4 and 6.2 log CFU/g (Fig. 3). The mean reduction in total aerobic bacteria was 0.4 ± 0.7 log CFU/g. A reduction in total aerobic bacteria after the ultrasound-steam treatment was observed in 77% of the samples, while an increase was observed in 23% of the samples. In 15% of the treated samples, the total aerobic bacterial count was above 5 log CFU/g, and in two treated samples, the count was above 6.0 log CFU/g. The highest value before treatment was 7 log CFU/g, and the lowest value after treatment was 3.5 log CFU/g. Total aerobic

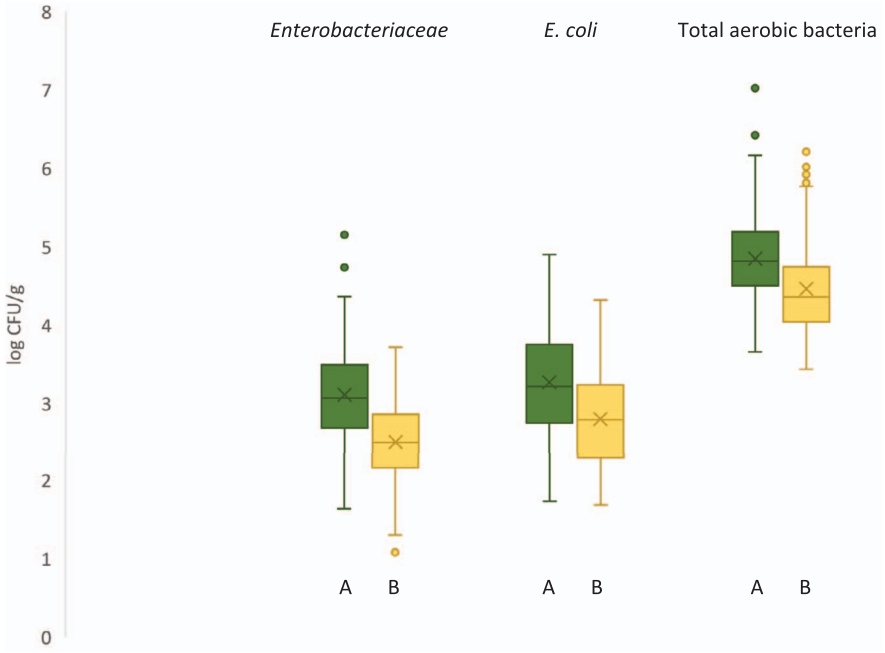

FIGURE 3. *Distribution of bacteria belonging to the* Enterobacteriaceae, E. coli, *and total aerobic bacteria on broiler neck skins before (A) and after (B) ultrasound-steam treatment.*

bacteria were found in all 103 samples both before and after treatment. The difference in numbers of total aerobic bacteria before and after treatment was highly significant ($P < 0.0001$).

Different treatment temperatures. Ten of the 58 samples where *C. jejuni* could be enumerated were treated at 84 to 85°C, and the remaining 48 samples were treated at 87 to 88°C. Of the 100 samples where *E. coli* and *Enterobacteriaceae* could be enumerated, 34 were treated at 84 to 85°C and 66 at 87 to 88°C. For total aerobic bacteria, 34 samples were treated at 84 to 85°C and 69 samples at 87 to 88°C. No significant difference in the reduction in any of the bacteria was observed between the different temperatures ($P = 0.1$ to 1.0).

Reduction of bacteria. Analysis of *C. jejuni* with simple linear regression showed that in general, there was a weak correlation ($R^2 = 0.27$) between the levels of *C. jejuni* bacteria on the neck skin samples before the treatment and the reduction achieved in this bacterial species (Fig. 4). The regression model resulted in a weak positive slope (0.2) that was statistically significant ($P = 0.04$, $R^2 = 0.08$). For the other parameters, the correlation was stronger ($R^2 = 0.7$, 0.6, and 0.6 for *Enterobacteriaceae, E. coli,* and total aerobic bacteria, respectively). The regression models for these groups resulted in a positive slope (0.6, 0.6, and 0.7, respectively) and were all highly significant ($P < 0.0001$; $R^2 = 0.4$, 0.3, and 0.7, respectively).

DISCUSSION

The reduction in *Campylobacter* achieved by the ultrasound-steam treatment in this study was not as high as reported in previous studies. In a Danish study, the mean reduction in *Campylobacter* on carcasses following ultrasound-steam treatment was around 1.0 log CFU/g *(20)*. The line speed in that slaughterhouse was, on average, 8,500 chickens per hour, while in our study, it was 18,000 chickens per hour. Higher line speed meant that the carcasses were exposed to the ultrasound-steam treatment for a shorter period. Other studies have indicated that longer treatment times result in higher reductions in bacteria *(2, 9, 19)*. Ultrasound-steam treatment for 5 s on the inside of the carcasses and 10 s on the outside has been found to result in a reduction in *Campylobacter* of around 2.5 log CFU/mL *(9)* or 2.5 log CFU per carcass *(2)*. The reductions in total viable bacteria also seem to be dependent on the duration of treatment, with, e.g., increased treatment time from 0.5 to 4 s on pork skin resulting in a final reduction in total viable bacteria of up to 3.3 log CFU/cm^2 *(19)*. Other studies examining steam pasteurization without the ultrasound component have also found a relationship between longer treatment times and higher reductions in bacteria *(15, 29)*.

The ultrasound-steam equipment can produce steam at up to 90 to 94°C. In this study, the temperature applied was either 84 to 85°C or 87 to 88°C, because the spices used later for some chicken products do not adhere properly to carcass skin treated at temperatures above 90°C. We did not find any significant difference in bacterial reductions between the two different treatment temperatures. In some cases, ultrasound-steam treatment at higher temperature has damaged the skin of the carcass, altering the product quality *(2, 20),* whereas in other cases, no visual changes have been detected *(9)*.

We found a weak correlation ($P = 0.04$) between the initial concentration of *Campylobacter* and the reduction achieved by treatment. However, this correlation was stronger ($P < 0.0001$) for the other bacteria studied (*Enterobacteriaceae, E. coli,* and total aerobic bacteria), probably because it is easier to reduce the amount of bacteria if the level is high from the beginning. Since the samples in our study were obtained after treatment by ultrasound-steam and air chilling, we cannot exclude the possibility that air chilling contributed to the reduction in bacteria. Other studies have shown varying results for the

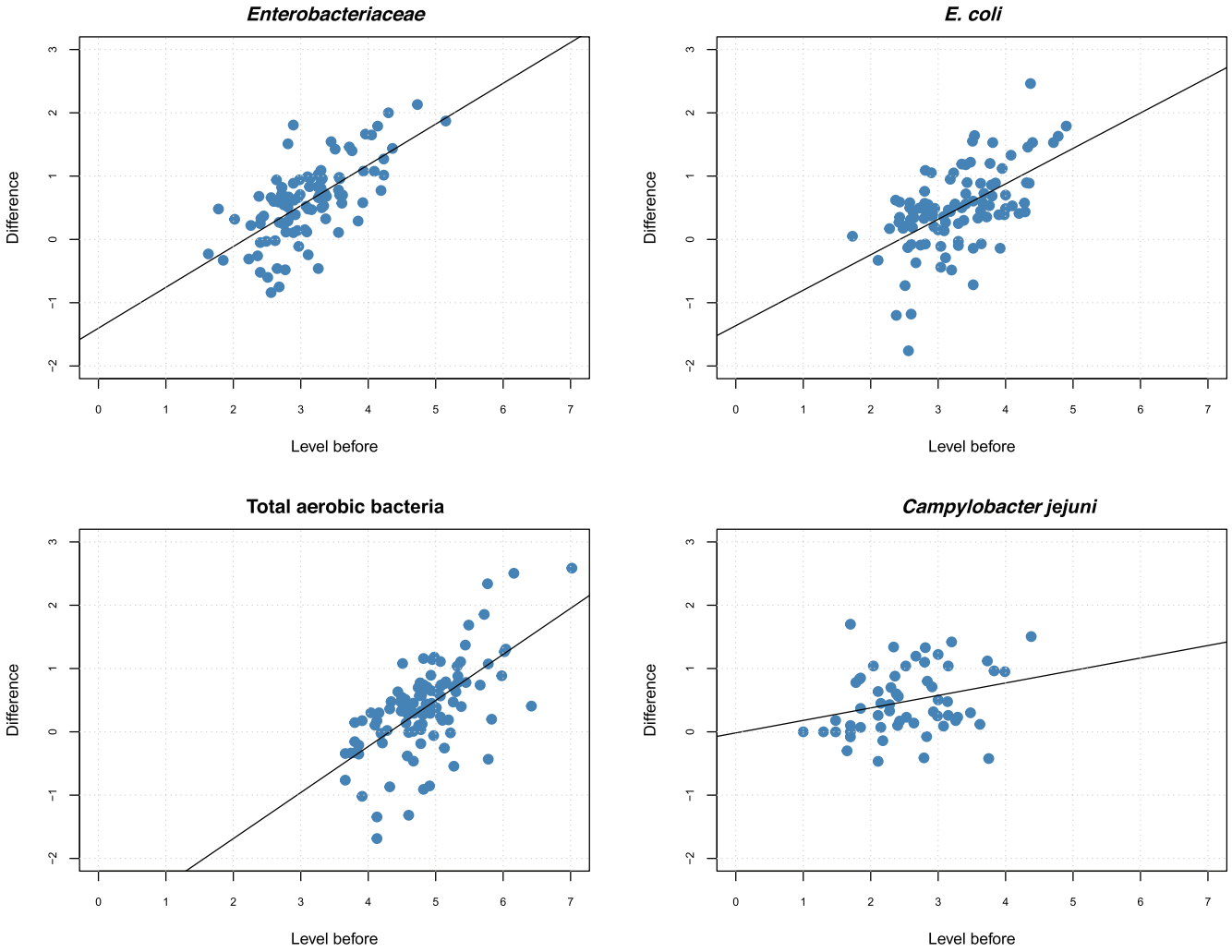

FIGURE 4. *Results of simple linear regression. The horizontal axis indicates the levels of* C. jejuni, Enterobacteriaceae, E. coli, *and total aerobic bacteria before the treatment, while the vertical axis shows the reductions (differences) in* C. jejuni, Enterobacteriaceae, E. coli, *and total aerobic bacteria numbers after the ultrasound-steam treatment.*

effect of air chilling on concentrations of bacteria. A significant reduction of 0.4 log CFU/g due to air chilling was found in one study *(2),* while another study found no significant effect of air chilling by itself without ultrasound-steam *(20)*.

The increase in *Campylobacter* observed in seven samples could be due to difficulties in enumeration of *Campylobacter* because the amounts of the other bacteria analyzed were reduced in these samples. Colonies of thermotolerant *Campylobacter,* especially *C. jejuni,* tend to spread, which results in difficulties in counting on some mCCDA plates because of swarming of *Campylobacter* colonies (ISO 10272-2 *(14)*). Another reason for the increase in *Campylobacter* observed in some samples could be variation in oxygen levels during transport, as *Campylobacter* spp. are microaerophilic and the atmosphere during transportation affects their survival. Although the reduction in *C. jejuni* was significant in this study, there were still large amounts of *Campylobacter* on the carcasses after treatment, with 12% of the samples having levels that exceeded 3 log CFU/g. It should be noted that the flocks chosen for this study were delivered from broiler producers that had previously often delivered chickens colonized with *Campylobacter* to slaughter. According to the process hygiene criterion for slaughter of broilers in the European Union *(4),* slaughterhouses have to ensure that the amount of *Campylobacter* bacteria on the neck skin does not exceed 3 log CFU/g, due to the risk of humans getting campylobacteriosis. However, in this study, we quantified the neck skins individually, whereas in the process hygiene criterion, a pool of at least 15 neck skins is analyzed. If the criterion is not fulfilled, the slaughterhouse must take action to improve slaughter hygiene, review process controls, identify the farm of origin, and review biosecurity measures on the farm of origin *(4)*.

This study examined full-scale ultrasound-steam treatment of broiler carcasses naturally contaminated with bacteria and found that the treatment achieved a significant reduction ($P < 0.0001$) in bacteria, but the levels of bacteria left on the carcasses after treatment were still high. Further studies are needed to determine whether the effectiveness of ultrasound-steam treatment is dependent on the duration of treatment and/or the temperature of the steam. In a One Health perspective, optimal measures must be implemented

at slaughter to reduce the numbers of food spoilage bacteria and pathogenic bacteria.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the slaughterhouse that participated in the study. The authors also thank the Ivar and Elsa Sandbergs Research Foundation for funding the project.

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