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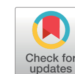
Reducing *Campylobacter jejuni*, *Enterobacteriaceae* and total aerobic bacteria on transport crates for chickens by irradiation with 265-nm ultraviolet light (UV–C LED)



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ABSTRACT

It is critical to maintain low levels of microbes in the whole food production chain. Due to high speed of slaughter, lack of time, and structural characteristics of crates, sufficient cleaning and disinfection of crates used for transporting chickens to abattoirs is a challenge. Inadequately cleaned transport crates for broiler chickens caused a major outbreak of campylobacteriosis in Sweden in 2016–2017, when the contaminated crates introduced *Campylobacter* to the chickens during thinning. This study evaluated the antibacterial efficacy of 265-nm ultraviolet (UV–C) LED light on artificially contaminated chicken transport crates. In a laboratory study, a transport crate artificially contaminated with *Campylobacter* and cecum contents was irradiated with 265-nm UV-C light by a continuous LED array in a treatment cabinet. The transport crate was sampled 52 times by cotton swabs before and after UV-C treatment for 1 min (20.4 mJ/cm^2) and 3 min (61.2 mJ/cm^2). The swab samples were analysed for *Campylobacter jejuni (C. jejuni)*, bacteria belonging to the family *Enterobacteriaceae*, and total aerobic bacteria. After irradiation with UV-C LED light for 1 min, a mean reduction in *C. jejuni* of log 2.0 ± 0.5 CFU/mL was observed, while after irradiation for 3 min the reduction was log 3.1 ± 1.0 CFU/mL. The mean reduction in *Enterobacteriaceae* was log 1.5 ± 0.3 CFU/mL after 1 min of irradiation and log 1.8 ± 0.8 CFU/mL after 3 min. The mean reduction in total aerobic bacteria was log 1.4 ± 0.4 CFU/mL after 1 min of irradiation and log 1.6 ± 0.5 CFU/mL after 3 min. Significant reductions in bacterial load were observed in all samples after UV-C treatment and extending the treatment time from 1 to 3 min significantly increased the reduction in *C. jejuni*. However, before implementation of UV-C LED treatment in commercial chicken abattoirs, the irradiation unit would need to be extended and/or the washing procedure before UV-C treatment, to reduce the amount of organic matter on transport crates, would need to be improved.

1. Introduction

Campylobacteriosis is the most commonly reported bacterial gastrointestinal disease in humans in the United States, Australia, Sweden, and other European countries. The most important risk factor for humans contracting campylobacteriosis is consumption of contaminated chicken products (Australian Government Department of Health, 2019; Center for Disease Control and Prevention, 2019; EFSA, 2017). It is critical to maintain low levels of microbes in the whole food production chain. Proper cleaning and disinfection is essential during the process, to reduce the occurrence of pathogenic bacteria on production animals, on food products, and on surfaces. This is of great importance, since cleaning and disinfection shortcomings have been shown to cause food poisoning. In one example, contaminated chicken transport crates caused a large outbreak of campylobacteriosis in Sweden in 2016–2017. The contaminated crates introduced *Campylobacter* to the chickens during thinning, when part of the flock was delivered to slaughter (Lofstedt, 2019). Studies show that pathogenic bacteria such as *Campylobacter* spp. and *Salmonella* spp. can remain on chicken transport crates even after cleaning and disinfection (Atterbury, Gigante, Tinker, Howell, & Allen, 2020; Hansson, Ederoth, Andersson, Vågsholm, & Engvall, 2005; Northcutt & Berrang, 2006; Peyrat, Soumet, Maris, & Sanders, 2008; Slader et al., 2002). Bacteria belonging to the family *Enterobacteriaceae* and total aerobic bacteria can be used to assess general cleanliness and to detect pathogenic bacteria at the abattoir (Haughton et al., 2011; Roccato et al., 2018). Various

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pathogens, total aerobic bacteria, coliforms, and *E. coli* have been analysed to evaluate the efficacy of different disinfection methods on transport crates for chickens (Atterbury et al., 2020; Berrang & Northcutt, 2005; Hinojosa et al., 2018; Northcutt & Berrang, 2006).

The demand for innovative disinfection approaches in the food industry is increasing (Morales-de la Peña, Welti-Chanes, & Martín-Belloso, 2019). There are alternative methods based on physical disinfection, such as ultraviolet light (UV). The forms UV-A (400–320 nm) and UV-B (320–290 nm) appear naturally as solar radiation, while UV-C radiation (290–100 nm) occurs mainly in artificial light (Giordano & Romano, 2015). It is known that UV-C light induces DNA damage in bacterial cells (Cheigh, Park, Chung, Shin, & Park, 2012). Although disinfection by conventional 254-nm UV-C light can be used to reduce the amount of *Campylobacter* (Haughton et al., 2011; Haughton, Lyng, Cronin, Fanning, & Whyte, 2012; Isohanni & Lyhs, 2009), the germicidal effect should increase at a wavelength of 265 nm (Kowalski, 2009). Comparison of the germicidal effect of 265 and 280-nm LEDs against *E. coli* in petri dishes showed that 265 nm was more efficient (Li, Wang, Huo, Lu, & Hu, 2017). However, to the best of our knowledge, there has been no research on the inactivating effect of 265-nm UV-C light on *Campylobacter*.

The poultry industry is calling for alternative approaches for disinfecting transport crates for chickens, since the cleaning and disinfection methods currently used are insufficient to consistently reduce *Campylobacter* and other bacteria. The aim of this study was to evaluate whether 265-nm UV-C LED light can be used to reduce *Campylobacter jejuni (C. jejuni)*, *Enterobacteriaceae*, and total aerobic bacteria on transport crates for chickens.

2. Materials and methods

2.1. LED light source

The UV unit used in the study was a WiSDOM DS (LED TAILOR INNOVA7ION, Salo, Finland) consisting of light-emitting diodes (LEDs), housed in an enclosed steel cabinet with external dimensions 615 mm × 445 mm × 330 mm and internal dimensions 500 mm × 325 mm × 140 mm (length × width × height) (Fig. 1). The internal dimensions represented the maximum size of the object to be irradiated. The object was placed in the middle of the cabinet, on a glass shelf at a distance of approximately 140 mm from the LEDs, which were installed in the roof and floor of the cabinet. Full 360° irradiation was ensured by the placement of the LEDs and reflective surfaces inside the cabinet. Samples were irradiated with a continuous LED array (Crystal IS, NY, USA) with wavelength 260–270 nm, an emission peak of 265 nm, and 12-nm bandwidth at full-width half maximum. The heat produced by the complete light system was low and had no effect on the test samples exposed to the UV-C light inside the cabinet. UV-C intensity was measured with a Gigaherz-Optik UV 3719–4 optometer (Gigahertz, Germany). The UV-C intensity was on average 0.34 mW/cm^2 (± 0.06 mW/cm^2) inside the cabinet, with good uniformity across the whole irradiated area. The energy received by a sample was 20.4 mJ/cm^2 (± 3.6 mJ/cm^2) when irradiated for 1 min and 61.2 mJ/cm^2 (± 10.8 mJ/cm^2) when irradiated for 3 min. The irradiation cabinet was equipped with a time control and safety features necessary when working with high-intensity UV-C light.

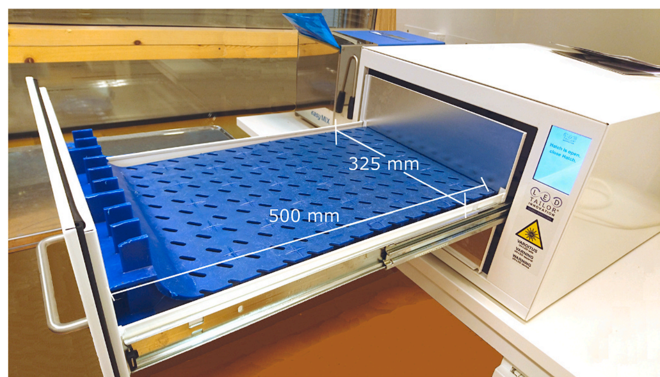

Fig. 1. Part of a transport crate in the UV-C irradiation cabinet (WiSDOM DS, LED TAILOR INNOVA7ION, Salo, Finland). Internal dimensions of the cabinet are indicated.

2.2. Transport crates

A cleaned used chicken transport crate (Linco food systems, Trige, Denmark) of the material high density polyethylene was donated by an abattoir in Sweden. The upper surface of the crate was relatively smooth but contained small scratches. It was also composed of multiple holes (18 mm length × 7 mm width) to facilitate ventilation and removal of feces during transport, and feces and water during cleaning (Fig. 1). The crate was cut into four pieces with dimensions 480–500 mm × 240–300 mm (length × width) to fit into the LED cabinet (Fig. 1). The upper surface of each of the pieces was measured and divided by a line into two equal parts. One piece of the crate at a time was submerged in a plastic box containing a mixture of chicken cecum contents and *C. jejuni* (see section 2.3). A lid was placed on the plastic box and the contents were mixed by tilting the box from side to side 10 times, after which it was left to stand for 10 min. The crate was then removed from the box and any visible caecal material on the surface of the crate was removed. The caecal mixture on the surface of the crate was evenly distributed with a sterile cotton swab pre-moistened in the caecal mixture. Samples were then taken by gloved hand, using a sterile cotton swab measuring 10 cm × 10 cm (Wellkang Ltd. t/a Wellkang Tech Consulting Suite B, London, UK) moistened with 30 mL buffered peptone water (BPW) (Oxoid CM0509; Basingstoke, UK). When swabbing the first part of the upper surface, the entire area was swabbed with overlapping horizontal and vertical strokes. The cotton swab was then placed in a sterile plastic bag, 90 mL BPW was added, and the suspension was homogenized in a stomacher for 1 min at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA). This sample served as an untreated control of the number of bacteria on the crates (A-samples). The crate was then placed in the LED cabinet and treated by UV-C light for 1 or 3 min. After UV-C treatment, the other part of the crate was sampled (B-sample) in exactly the same way as the control. A total of 52 untreated (A) + 52 treated (B) samples were collected from the crate surfaces, 25 (A) + 25 (B) after treatment with UV-C light for 1 min and 27 (A) + 27 (B) after treatment with UV-C light for 3 min.

2.3. Bacteriological analyses

2.3.1. Quantification of C. jejuni

Analysis for *C. jejuni* was performed according to ISO 10272–2 (2017). Briefly, a 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared. To ensure the possibility to estimate low numbers of *Campylobacter,* 1 mL from the initial suspension was distributed on the surface of four regular (90 mm) plates of modified charcoal cephoperazone desoxycholate agar (mCCDA) (Oxoid, Basingstoke, UK). For the other dilutions, 0.1 mL was surface plated onto each mCCDA plate. The plates were incubated at 41.5 ± 0.5 °C for 44 ± 4 h in a jar with microaerobic atmosphere generated by use of CampyGen™ (Oxoid, Basingstoke, UK). A blood agar plate (National Veterinary Institute (SVA), Uppsala, Sweden) with a *C. jejuni* strain (CCUG 43594) was also placed in each jar, as a positive control of the microaerobic atmosphere. After incubation, colonies characteristic of *C. jejuni* were quantified and the number of *Campylobacter* was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.3.2. Quantification of bacteria belonging to the family Enterobacteriaceae

Analysis for bacteria belonging to the family *Enterobacteriaceae* was performed according to NMKL 144 (3rd Ed. 2005). The previously prepared 10-fold dilutions were also used to estimate counts of *Enterobacteriaceae* in samples. From each dilution, 1.0 mL was mixed carefully with 10–15 mL violet red bile glucose agar (VRBG) (Becton, Dickinson and Company, Sparks USA) in a Petri dish and left to solidify, and then an overlay of 5 mL VRBG was added. Plates were then incubated at 37 ± 1 °C for 24 ± 2 h. Bacterial counts were performed on plates with 15–150 colonies. Five colonies preliminarily identified as *Enterobacteriaceae* were cultured on blood agar and incubated at 37 ± 1 °C for 24 ± 2 h. Presence of bacteria belonging to the family *Enterobacteriaceae* was confirmed by oxidase test and the number of *Enterobacteriaceae* was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.3.3. Quantification of total aerobic bacteria

Total aerobic bacteria was quantified according to NMKL 86 (5th Ed. 2013). From the initial dilution series prepared for each sample, 1.0 mL aliquots of each dilution were mixed with 15–20 mL of plate count agar (PCA) (Oxoid, Basingstoke, UK) and left to solidify, and then an overlay of 5–10 mL PCA was added. Plates were then incubated at 30 ± 1 °C for 72 ± 6 h. Bacterial counts were performed on plates with 25–250 colonies and total aerobic bacteria was expressed as log CFU per mL. The detection limit was log 1.0 CFU/mL.

2.4. Simulation of inadequately cleaned transport crates

Intestinal contents from 10 to 15 broiler caeca from *Campylobacter*-negative flocks according to the Swedish Campylobacter program (Hansson et al., 2007) were used to simulate caecal contamination of transport crates. The caeca were stored at −20 °C and thawed by storage in the refrigerator the day before analyses. The caeca were cut into 1–2 cm pieces using sterile scissors and tweezers, placed in a bottle with 0.5 L BPW, a lid was added, and the bottle was shaken. The contents were transferred to sterile plastic bags and homogenized for 1 min at 240 rpm in a stomacher. The contents of the bags were then poured into a clean and disinfected 40 L plastic box. An additional 5 L BPW and 40 mL of an overnight culture of *C. jejuni* (CCUG 43594) in brain heart infusion broth (BHI) (CM1135; Oxoid. Basingstoke, United Kingdom) were poured into the plastic box.

2.5. Initial concentrations of C. jejuni, bacteria belonging to the family Enterobacteriaceae, and total aerobic bacteria

The initial concentrations of bacteria in the caecal mixture containing chicken caeca, the overnight culture of *C. jejuni*, and BPW were quantified as described in section 2.3. Mean concentration of *C. jejuni*, *Enterobacteriaceae* and total aerobic bacteria in the caecal mixture were log 5.3 ± 0.5 CFU/mL, log 5.2 ± 0.3 CFU/mL, and log 6.1 ± 0.5 CFU/mL respectively (Fig. 2).

2.6. Statistical analyses

The data obtained in the study were compiled and analysed using Microsoft Office Excel and R studio (RStudio® version 1.2.1335 - Windows 7+). Bacterial counts (CFU/mL) were $\log_{10}$ transformed. Standard deviations of bacterial reductions following 1 and 3 min treatments were calculated. Statistical significance was determined by the paired *t*-test, which was performed for both treatment durations for each of the three bacterial groups under investigation. The Welch two-sample *t*-test was conducted to determine significant differences between treatment times. Differences before and after treatment, and between treatment times, were deemed significant at $p < 0.05$.

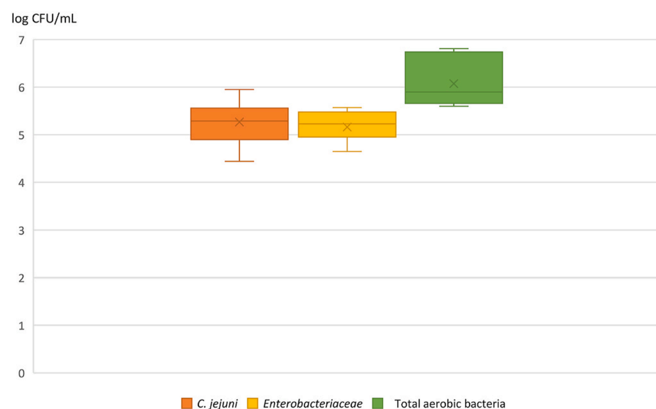

Fig. 2. Initial concentrations of *C. jejuni*, members of the family *Enterobacteriaceae*, and total aerobic bacteria in the mixture used to simulate caecal contamination of inadequately cleaned transport crates in the 52 analyses.

3. Results

3.1. Quantification of C. jejuni

The transport crate was irradiated 52 times and reductions in the number of *C. jejuni* were observed in all swab samples. Following irradiation of transport crate sections with UV-C light for 1 min, the mean reduction in *C. jejuni* was log 2.0 ± 0.5 CFU/mL. The concentration varied between log 4.4 and 5.8 CFU/mL before treatment, and between log 2.2 and 3.8 CFU/mL after treatment. A significant increase in the reduction of *C. jejuni* was observed on extending the treatment time from 1 to 3 min. In the treatment where the transport crates were irradiated with UV-C light for 3 min, the mean reduction in *C. jejuni* was log 3.1 ± 1.0 CFU/mL. The concentration varied between log 4.5 and 7.1 CFU/mL before treatment, and between log 2.0 and 3.8 CFU/mL after treatment, in that case (Fig. 3). The difference in the numbers of *C. jejuni* on the crate before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

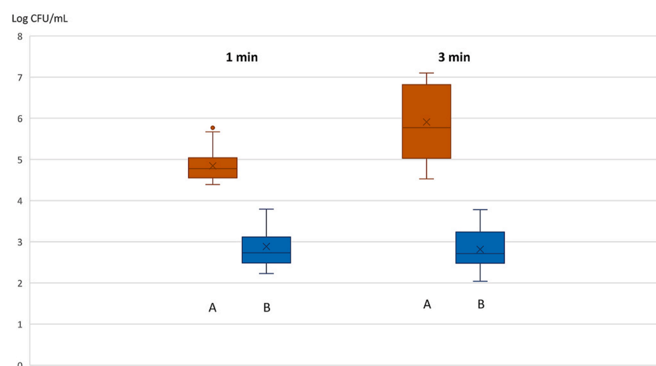

Fig. 3. Concentration of *C. jejuni* in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

3.2. Quantification of bacteria belonging to the family Enterobacteriaceae

The transport crate was irradiated 52 times and reductions in bacteria belonging to the family *Enterobacteriaceae* were observed in all swab samples. Countable numbers were present after treatment in all samples except one, in which the amount was reduced below the limit of detection after 3 min of irradiation. This value is excluded from Fig. 4. Following treatment of transport crate sections with UV-C light for 1 min, the mean reduction in *Enterobacteriaceae* was log

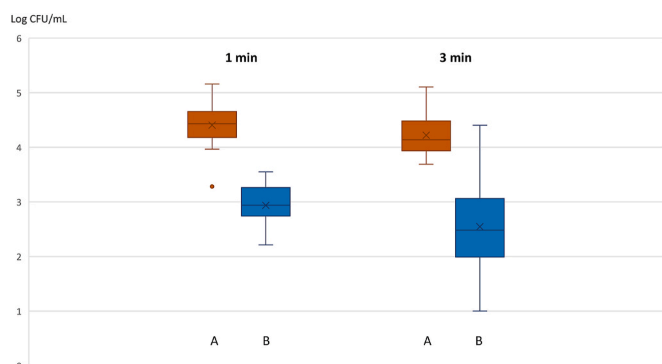

Fig. 4. Concentration of bacteria belonging to the family *Enterobacteriaceae* in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

1.5 ± 0.3 CFU/mL. The concentration varied between log 3.3 and 5.2 CFU/mL before treatment, and between log 2.2 and 3.6 CFU/mL after treatment.

No significant increase in the reduction in *Enterobacteriaceae* was observed on extending the treatment time from 1 to 3 min. Following irradiation with UV-C light for 3 min, the mean reduction in *Enterobacteriaceae* was log 1.8 ± 0.8 CFU/mL. The concentration varied between log 3.7 and 5.1 CFU/mL before treatment, and between log 1 and 4.4 CFU/mL after treatment (Fig. 4). The difference in the number of bacteria belonging to the family *Enterobacteriaceae* on the crate before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

3.3. Quantification of the total aerobic bacteria

The transport crate was irradiated 51 times and reductions in the total aerobic bacteria were observed in all swab samples, while one sample was excluded due to contamination. The number of bacteria present was countable in all samples subjected to the treatment at both treatment times. After treatment of the crate with UV-C light for 1 min, the mean reduction in total aerobic bacteria was log 1.4 ± 0.4 CFU/mL. The concentration varied between log 5.2 and 6.2 CFU/mL before treatment, and between log 3.8 and 4.6 CFU/mL after treatment. No significant increase in the reductions in total aerobic bacteria was observed on extending the treatment time from 1 to 3 min. In the treatment where the crate was irradiated with UV-C light for 3 min, the mean reduction in total aerobic bacteria was log 1.6 ± 0.8 CFU/mL. The concentration varied between log 4.9 and 5.5 CFU/mL before

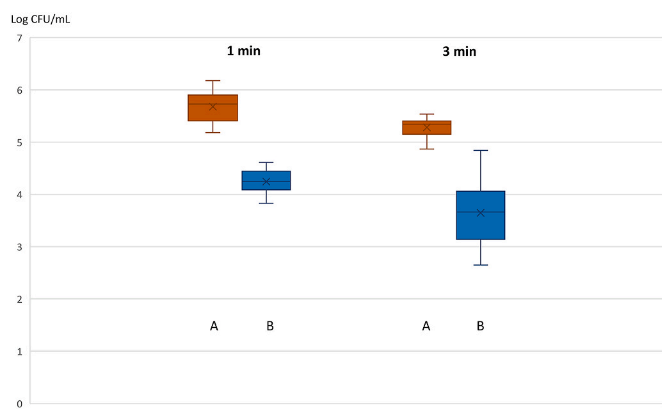

Fig. 5. Concentration of total aerobic bacteria in swab samples from an artificially caeca-contaminated chicken transport crate taken: (A) before treatment and (B) after irradiation with UV-C light for 1 or 3 min.

treatment, and between log 2.7 and 4.8 CFU/mL after treatment (Fig. 5). The difference in total aerobic bacteria before and after treatment with UV-C light was highly significant ($P < 0.0001$) for both treatment times (1 min and 3 min).

4. Discussion

Significant reductions in bacterial numbers were observed in all samples after irradiation of the artificially caeca-contaminated transport crate sections with 265-nm UV-C light. However, the bacterial reductions observed were lower than those reported in other studies examining the effect of 265-nm UV-C light on other bacteria and materials, e.g., suspensions of *Salmonella* spp. and *Staphylococcus aureus* in petri dishes (Lee, Yoon, Park, & Ryu, 2018) and *E. coli* in petri dishes (Li et al., 2017). In the present study, total inactivation of *C. jejuni* was not achieved. One explanation could be a "shadow" effect caused by the holes and cracks in the crate, in which the bacteria could have been protected from irradiation (Hinojosa et al., 2018). The upper surface of the crate was not completely smooth because it was composed of multiple holes for ventilation and removal of feces. Additionally, the surface consisted of small scratches from the claws of the chickens due to its previous use. The "shadow" effect has been observed in experiments performed on chicken meat (Haughton et al., 2011; Haughton, Lyng, Cronin, Fanning, & Whyte, 2012). Another reason for the failure to achieve total inactivation of *C. jejuni* could have been that the caecal mixture in which the crate sections were submerged was opaque and UV-light has low penetrating capacity (Ninios, Lundén, Korkeala, & Fredriksson-Ahomaa, 2014).

It has been observed that the antibacterial efficacy of UV irradiation treatment may depend on the initial concentration of bacteria (Isohanni & Lyhs, 2009). In that case, the reduction in bacteria might have been different if a lower concentration of bacteria had been used to simulate inadequately cleaned transport crates in the present study. Interestingly, the initial number of bacteria on the transport crate was at the same level or lower than that found on naturally contaminated transport crates from an abattoir in the UK (Atterbury et al., 2020).

Extending the treatment time from 1 to 3 min resulted in greater reductions in *C. jejuni* of log 1.1 CFU/mL being observed, which was statistically significant ($p < 0.05$). However, considerable numbers of *C. jejuni* were still present on the transport crates after the 3 min treatment (log 2.0–3.8 CFU/mL). No significant differences were observed in *Enterobacteriaceae* or total aerobic bacteria when 1 and 3 min treatments were compared. This might be due to *Campylobacter* being more sensitive to UV-light than other Gram-negative bacteria associated with poultry, such as *E. coli* and *Salmonella* Enteritidis (Haughton et al., 2011; Murdoch, Maclean, MacGregor, & Anderson, 2010). In the 3 min treatment, the standard deviation in counts was higher for *C. jejuni* than for *Enterobacteriaceae* and total aerobic bacteria. Fluctuations in the amount of *Campylobacter* have been observed previously, leading to the suggestion that *Campylobacter* is less robust to environmental conditions than the other bacteria tested (Atterbury et al., 2020).

As treatments to reduce *Campylobacter*, conventional 254-nm UV-C light and 405-nm blue light have been studied. In studies where chicken meat was irradiated with 254-nm UV-C light for less than 1 min, the reduction in *Campylobacter* was only log 0.6–0.8 CFU/g (Haughton et al., 2011; Isohanni & Lyhs, 2009). In another study, micro-plates inoculated with *Campylobacter* and irradiated with 405-nm blue light did not show any significant reductions when treated for less than 5 min (Murdoch et al., 2010). This indicates that short treatment times using conventional 254-nm UV-C light and 405-nm blue light may not be sufficient to achieve acceptable reductions in *Campylobacter*.

Introducing UV-C light as a disinfection method at a commercial abattoir could be a challenge, due to the high speed of slaughter and the humidity of the crates. In one of the largest abattoirs in Sweden, around 50 million broilers are slaughtered per year, equivalent to five broilers

per second. This means that the rate of cleaning and disinfection of transport crates also has to be very high and that there is just a limited time for any disinfection procedure. Disinfection of the crates is currently performed after the cleaning process, and the treatment only takes a few seconds.

When using UV light for disinfection of objects, the energy received by a sample depends on treatment time and distance from the light source. In the present study, only 1 min was needed to reduce *C. jejuni* by 2 log CFU/mL (0.02 J/cm^2). To reduce *C. jejuni* by 2.3 log CFU/mL on micro-plates with 405-nm blue light, 25 min (15 J/cm^2) was needed in a previous study (Murdoch et al., 2010). Levels of *C. jejuni* can be reduced below the limit of detection when packaging materials and food contact surfaces are irradiated for less than 1 min with 254-nm UV-C light with similar energy dosage to that used in the present study (Haughton et al., 2011). However, when porous material (polyethylene-polypropylene) was tested in that study, the energy dose had to be increased to reduce *C. jejuni* below the limit of detection. Since chicken transport crates have a porous surface, this suggests that more LED diodes should be added to the UV unit to increase the energy exerted on the samples. This could be a way to achieve greater reductions in bacteria without having to increase the treatment time.

To enable longer treatment times under commercial conditions, a longer tunnel lined with UV-C LED lights, through which the crates would pass during 1 min, could be built. However, this might be a costly investment for the abattoir and not practically possible in a commercial setting. Another solution could be to reduce the amount of fecal matter, and thereby decrease the initial concentration of bacteria on the crates, by improving the washing procedure prior to UV-C treatment (Atterbury et al., 2020). Alternatively, the efficiency of the UV-C unit could be improved by decreasing the distance between the transport crate and the light source (Haughton, Grau et al., 2012; Lee et al., 2018) or by adding more LED diodes.

5. Conclusions

Disinfection by UV-C light is not a new technology, but to our knowledge it has not been used previously for cleaning and disinfection of transport crates for chickens. Evaluation of the antibacterial efficacy of 265-nm UV-C LED light on artificially contaminated chicken transport crates in this study revealed significant reductions in *C. jejuni*, *Enterobacteriaceae*, and total aerobic bacteria. Irradiation treatment for 1 or 3 min effectively reduced *C. jejuni* on the crates, although considerable numbers of bacteria were still present on the crates after the treatment. Thus UV-C LED light may have good potential for reducing microbial loads on transport crates for chickens, but if UV-C light treatment is used in abattoirs, the UV unit would need to be extended and/or the washing process prior to UV-C treatment would need to be improved.

CRediT authorship contribution statement

Madeleine Moazzami: Conceptualization, Writing - original draft. **Lise-Lotte Fernström:** Formal analysis. **Ingrid Hansson:** Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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