

# The efficacy of pulsed ultraviolet light processing for table and hatching eggs

J. R. Cassar,<sup>\*</sup> L. M. Bright,<sup>\*</sup> P. H. Patterson,<sup>\*,1</sup> E. W. Mills,<sup>\*</sup> and A. Demirci<sup>†</sup>

<sup>\*</sup>Department of Animal Science, The Pennsylvania State University, University Park, PA 16802, USA; and

<sup>†</sup>Department of Agricultural and Biological Engineering, The Pennsylvania State University, University Park, PA 16802, USA

**ABSTRACT** In the United States, every year an average of 287.1 eggs are consumed per person, and over 14.1 billion eggs are set in hatchery incubators to produce chicks destined for the egg and meat bird industries. By reducing the microbial load on eggs, food-borne-associated outbreaks can be reduced while good chick health is maintained. Pulsed ultraviolet (PUV) light system delivers an energy-intense broad spectrum (100–1,100 nm) pulse derived from a xenon flashlamp. In recent years, PUV light has been shown to reduce microbial pathogens on the surface of shell eggs by using a static PUV light system. In this study, shell eggs were surface inoculated with *Escherichia coli* or *Enterococcus faecium* and treated with PUV light using a modified egg candling conveyor that provided complete rotation of eggs under a flashlamp. Pulsed UV light treatment inactivated both microbial strains, with greater energy resulting in a greater germicidal response ( $P < 0.05$ ). Treatments of

1.0, 2.4, 3.1, and 4.9 J/cm<sup>2</sup> resulted in microbial reductions (Log<sub>10</sub> CFU/cm<sup>2</sup>) of 3.83, 4.26, 4.28, and 4.62 for *E. coli* and 2.04, 3.12, 3.11, and 3.82 for *E. faecium*, respectively. This study also evaluated the effects of PUV light treatment of hatching eggs (commercial Leghorn hybrids) on both embryo and chick growth parameters. Using the same system, 4 replicates of 125 fertile eggs per rep were treated with 0 (control), 4.9, 24.4, or 48.8 J/cm<sup>2</sup> of PUV light. After processing, eggs were placed in a commercial incubator under normal incubation conditions. There was no significant effect of the PUV light treatment on percent fertility, hatchability, or hatch ( $P > 0.05$ ). Furthermore, there were no significant effects on posthatch observations, including livability and average bird weight at hatch or at 42 d of age ( $P > 0.05$ ). In conclusion, this study supports the application of PUV light as an effective antimicrobial intervention for both table and hatching eggs.

**Key words:** eggs, pulsed ultraviolet light, incubation, decontamination

2021 Poultry Science 100:100923  
<https://doi.org/10.1016/j.psj.2020.12.021>

## INTRODUCTION

The egg industry in the United States provides both table eggs for consumption and hatching eggs produced for maintenance of future poultry flocks. According to the National Agricultural Statistics Service, the United States produces approximately 99.1 billion table eggs per year (USDA, 2020) to accommodate the average individual consumption of 287.1 eggs annually (AEB, 2019). Furthermore, according to the National Agricultural Statistics Service, in 2019 approximately 14.1 billion eggs were utilized to produce broiler-type and layer-type chicks and replenish the poultry industry

needs (USDA, 2020). With the current scale of egg production, ensuring safety of consumers, producers, and animal welfare is crucial.

Between 2009 and 2015, the Centers for Disease Control and Prevention reported that table eggs accounted for 9% of total foodborne illnesses in the United States (Dewey-Mattia et al., 2018). *Salmonella enterica* serovars Typhimurium and Enteritidis are the major pathogens associated with egg-borne outbreaks. To ensure food safety during table egg processing, distribution, and consumption, eggs are primarily washed with a hot detergent solution and rinsed with a sanitizer solution (O'Bryan et al., 2017). A variety of sanitizers have been investigated including chlorine, hydrogen peroxide, electrolyzed oxidizing water, ozone, UV light, pulsed ultraviolet light, etc., resulting in a range of effectiveness, affordability, and accessibility (Keener, 2017; Vinayananda et al., 2017).

In addition to the food safety concerns of table eggs, decontamination of hatching eggs is also important for maintaining good chick health. When hatching, chicks

© 2020 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received September 17, 2020.

Accepted December 8, 2020.

<sup>1</sup>Corresponding author: [php1@psu.edu](mailto:php1@psu.edu)

are exposed to microorganisms present in the environment, including those on the surface of eggshells, some of which can be pathogenic. Pathogenic strains of *Escherichia coli* can cause significant poultry health problems that result in economic losses because of costs of containment, mortality, and carcass disposal (Shane and Faust, 1996; Mellata, 2013). Commercial decontamination of hatchery eggs is primarily achieved with use of chemical sanitizers (Williams, 1970; Shane and Faust, 1996). Numerous studies have investigated various interventions for decontamination of hatching eggs, including but not limited to chlorine dioxide foam (Patterson et al., 1990), alkylated pyrazines (Kusstascher et al., 2017), and UV light (Turtoi and Borda, 2014).

Pulsed ultraviolet (PUV) light has emerged as a novel technology that continues to prove effective as an antimicrobial intervention for food surfaces, including shell eggs. Pulsed UV light is termed as a result of the broad spectrum (100–1,100 nm) emitted from a xenon flashlamp that is delivered in a series of pulses (100 ns to 2 ms) with over 50% of the energy originating from the UV region (100–400 nm). The main germicidal mechanism of PUV light is the formation of DNA thymine dimers as a result of UV exposure. Secondary germicidal mechanisms are a result of longer wavelengths in combination with short pulses that produce localized heating and microvibrations, respectively, that contribute to microbial cell membrane collapse and death (Sonenshein, 2003; Krishnamurthy et al., 2010; Cassar et al., 2020). In addition to PUV light's antimicrobial effectiveness for numerous other foods, it has also been reported to achieve a significant reduction of *Salmonella* serotypes on the surface of eggshells (Hierro et al., 2009; Keklik et al., 2010; Lasagabaster et al., 2011) and in liquid egg (Ouyang et al., 2020).

Specific to eggshells, Keklik et al. (2010) inoculated a 2 cm<sup>2</sup> surface area of shell eggs with *Salmonella* Enteritidis before treatment with PUV light. By adjusting proximity and duration of exposure to PUV light, microbial reductions ranged from 1.3 to 7.7 Log<sub>10</sub> CFU/cm<sup>2</sup> after 0.8 to 35.3 J/cm<sup>2</sup>. Hierro et al. (2009) also investigated the decontamination of *Salmonella* Enteritidis on the exterior of washed and unwashed shell eggs. Eggs were inoculated by submersion then placed on a quartz glass window between 2 PUV light flashlamps for treatment. The microbial reduction ranged from 0.14 to 2.49 and 0.21 to 1.85 Log<sub>10</sub> CFU/cm<sup>2</sup> for washed and unwashed eggs, respectively, after 2 to 12 J/cm<sup>2</sup> of PUV light exposure. Using the same system as Hierro et al. (2009), Lagasabaster et al. (2011) inoculated the surface of shell eggs with *Salmonella* Typhimurium before PUV light treatment. After exposure to 2.1 J/cm<sup>2</sup> of PUV light, a 5.0 and 4.9 Log<sub>10</sub> CFU/cm<sup>2</sup> reduction was reported on the surface of washed and unwashed eggs, respectively. Ouyang et al. (2020) used PUV light to inactivate *Salmonella* Enteritidis inoculated in liquid egg white. After 45.6 J/cm<sup>2</sup> of PUV light exposure, a significant reduction of 1.98 Log<sub>10</sub> CFU/mL was reported.

These previous studies mentioned above document the significant reduction of *Salmonella* on the surface of shell eggs or liquid egg treated with PUV light. This study differs from previous research using a modified commercial egg candling system to convey and rotate eggs under a PUV light flashlamp. The design of the PUV light conveyor system used in this project is an example of how commercially processed eggs could be treated. The objectives of this experiment are 1) to further evaluate the germicidal response on the surface of shell eggs after treatment by pulsed ultraviolet light with the commercial egg conveyor and 2) to evaluate incubation and grow-out parameters of hatching eggs treated with the selected PUV light doses.

## MATERIALS AND METHODS

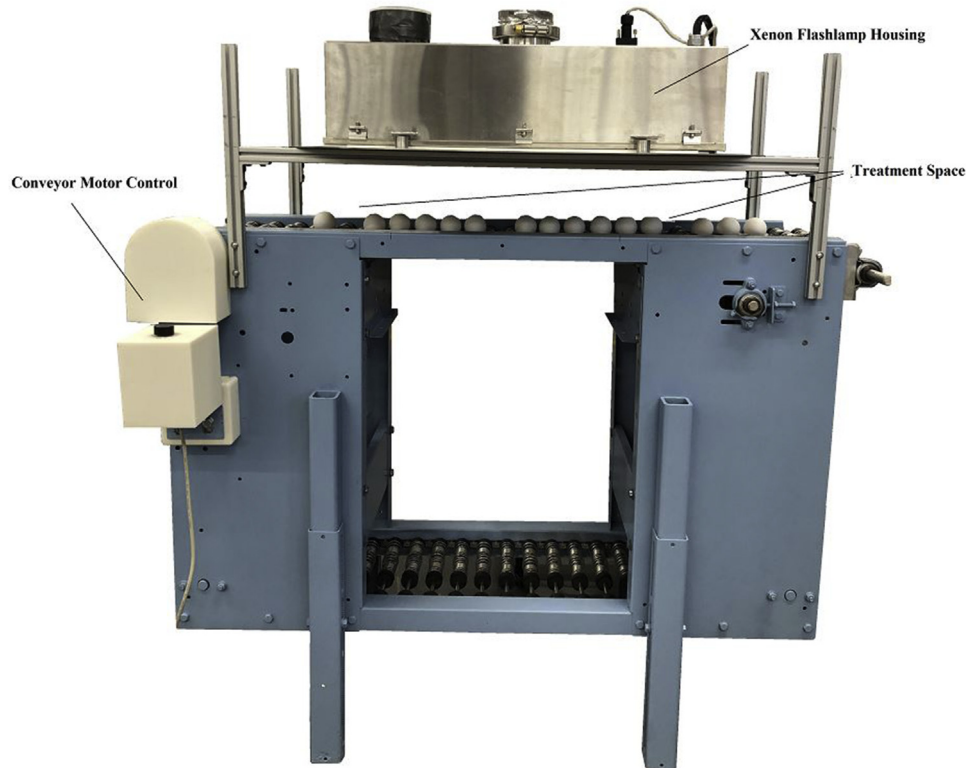
### *Pulsed Ultraviolet Light System*

Pulsed ultraviolet light was generated from a xenon flashlamp connected to a Pulsed Light RC-802 Interweave System (model Z-5000; Xenon Corporation, Wilmington, MA). Eggs were exposed to PUV light using a flashlamp positioned above a modified egg candling conveyor (Figure 1). The 128 cm long by 29 cm wide conveyor was fitted with adjustable braces to support a flashlamp housing and the 40.6 cm xenon gas flashlamp that was centered along the length of the conveyor. The conveyor had a fixed speed of 4.8 cm/s, which resulted in a complete 360° rotation along the equator of the egg approximately every 17 cm. Processing time for a single pass was 26.7 s. The lamp pulsed 3 times (360 μs/pulse) per second emitting a polychromatic spectrum (100–1,100 nm) with over 50% of the total energy deriving from the UV region (Sonenshein, 2003).

The total energy emitted from the PUV flashlamp was measured using a Nova Laser Power/Energy Monitor (P/N 1J06013, OPHIR Optronics Ltd., Wilmington, MA) with a 46 mm aperture pyroelectric metallic absorber (P/N 1Z02860, OPHIR Optronics Ltd.). The power monitor measured the total energy that was delivered across a single plane within the treatment space. To account for the constant rotation of the eggs, the total energy measured was divided in half because only one half of any given egg was exposed to PUV light at any given time on the conveyor.

### *Eggs*

The eggs used in both the microbial response and hatchability portions of this study were provided by the Hy-Line North America hatchery (Elizabethtown, PA) and produced by 48-week-old Lohmann LSL Light hens. Eggs weighed on average was 60.2 ± 2.0 g and had an average surface area of 71.5 ± 3.8 cm<sup>2</sup>, which falls within the classification of "Large" eggs (USDA, 2000). Surface area was calculated using a modeling system described by Troscianko (2014). Eggs were not altered in any way before treatment, though eggs with visible surface debris were excluded from the study.



**Figure 1.** Modified egg candling conveyor fixed with a xenon PUV light flashlamp. Abbreviation: PUV, pulsed ultraviolet.

## Microorganisms

*E. coli* K12 and *Enterococcus faecium* (NRRL B-2354) were selected as nonpathogenic model microorganisms as supported by previous research (Boney et al., 2018; Cassar et al., 2019, respectively). *E. coli* K12 was obtained from the *E. coli* Reference Center at the Pennsylvania State University (University Park, PA). Naladixic acid and streptomycin sulfate antibiotic resistant (NSR) cultures were prepared as described by Catalano and Knabel (1994), and a working culture of *E. coli* K12-NSR was derived from a frozen culture prepared by Cassar et al. (2019). Cultures were maintained in tryptic soy broth (BD-Difco, Franklin Lakes, NJ) supplemented with 0.6% of yeast extract and 100 mg/mL of both nalidixic acid and streptomycin sulfate (TSBYE-NS). *E. coli* K12-NSR was plated on tryptic soy agar (BD-Difco) supplemented with 0.6% of yeast extract and 100 mg/mL of both nalidixic acid and streptomycin sulfate. Working cultures were subcultured every 14 d and maintained in TSBYE-NS at 4°C.

*E. faecium* (NRRL B-2354) was obtained from the Pennsylvania State University Food Science Culture Collection. Stock cultures were prepared and kept at cryopreservation conditions (−80°C) in sterile Brain Heart Infusion broth (DOT Scientific, Burton, MI) with 20% glycerol. Stock cultures were aseptically transferred to tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and incubated for 24 h at 37°C to create a working culture. *E. faecium* was plated on

m-Enterococcus agar (Neogen; Lansing, MI). Working cultures were maintained in TSBYE at 4°C and subcultured every 14 d.

## Inoculum Preparation and Inoculation

An inoculum of *E. coli* K12-NSR was prepared as described by Cassar et al. (2019). The *E. coli* K12-NSR working cultures were transferred into 1,000 mL TSBYE-NS and incubated at 37°C for 24 h. For *E. faecium* inoculum, working cultures were transferred to 1,000 mL of TSBYE and incubated at 37°C for 24 h. After incubation, *E. coli* K12-NSR and *E. faecium* working cultures were centrifuged at 10°C and  $3,330 \times g$  for 30 min, the supernatant was removed, and 1,000 mL of sterile buffered peptone water (BPW; BD) was used to resuspend the pellet, yielding  $10^8$  to  $10^9$  Log<sub>10</sub> CFU/mL. Eggs were removed from cooler storage 6 h before being inoculated to reach room temperature (ca. 20°C). Fifteen eggs were placed into a sterile plastic container positioned in a rotating water-bath (Precision, Winchester, VA) at 100 rpm with room temperature water (ca. 20°). One thousand milliliter of either *E. coli* K12-NSR or *E. faecium* inoculum was added to the containers with 15 eggs each. Eggs, submersed for 10 min, were then aseptically transferred to an incubator (37°C) for 30 min to allow surface drying and contribute to microbial surface attachment, resulting in a  $10^4$  to  $10^5$  Log<sub>10</sub> CFU/cm<sup>2</sup> population.



## Pulsed Ultraviolet Light Treatment System

**Table Eggs** Inoculated eggs were held for 30 min at ca. 37°C to dry the egg surface and contribute to microbial attachment before PUV light treatment. The flashlamp was positioned parallel to the long axis of the conveyor at 9.5, 14.5, and 19.5 cm above the surface of the eggs to deliver 4.9, 3.1, and 2.4 J/cm<sup>2</sup>, respectively. The flashlamp was also positioned perpendicular to the long axis of the conveyor at 14.5 cm above the surface of the eggs to deliver 1.0 J/cm<sup>2</sup>. Four treatment levels were used with 15 eggs in each treatment exposed to a total energy of 1.0, 2.4, 3.1, and 4.9 J/cm<sup>2</sup> of PUV light, respectively. Immediately before and following PUV light treatment, the surface temperature of the eggs was measured using a noncontact infrared thermometer (Lasergrip 800; Etekcity, Anaheim, CA).

**Fertile Eggs** Using the same modified egg candling conveyor, the xenon flashlamp was positioned 9.5 cm above and parallel to the long axis of the conveyor. At this setting, eggs were passed under the PUV light 1, 5, or 10 times in series to deliver 3 energy treatments of 4.9, 24.4, and 48.8 J/cm<sup>2</sup>, respectively. A total of 2,000 fertile eggs were used in this phase of the study divided equally into 4 replicates per treatment level (n = 125 eggs/rep) for PUV light treatments of 0 (control), 4.9, 24.4, and 48.8 J/cm<sup>2</sup>. Similar to table eggs, fertile egg surface temperatures were recorded following treatment.

## Microbial Analysis of Table Eggs

*E. coli* K12-NSR and *E. faecium* inoculated eggs were treated separately when evaluating the impact of PUV light on microbial reduction of eggs. At each treatment level, eggs (n = 15) were evaluated for both *E. coli* K12-NSR and *E. faecium*. Following treatment, eggs were aseptically transferred to a filtered stomacher bag (Classic 400, Seward Ltd., Worthing, UK), with 70 mL of BPW (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK). Samples were then vigorously shaken by hand for 30 s before being serially diluted in BPW. *E. coli* K12-NSR and *E. faecium* samples were plated on tryptic soy agar supplemented with 0.6% of yeast extract and 100 mg/mL of both nalidixic acid and streptomycin sulfate and m-Enterococcus agar plates, respectively, using an autoplate (Autoplate 4000; Spiral Biotech, San Diego, CA) and incubated at 37°C for 24 h. Microbial reductions (Log<sub>10</sub> CFU/cm<sup>2</sup>) were determined by comparing CFU counts of treated samples to the CFU counts of controls (untreated samples). Natural microflora, if present, would have been accounted for when comparing treated samples to controls. For samples that resulted in zero colonies, enrichment was performed by transferring 1 mL from the BPW rinse solution to a 9 mL solution of TSBYE-NS and TSBYE for *E. coli* K12-NSR and *E. faecium*, respectively. After incubation for 24 h at 37°C, positive enrichment was determined by the presence of microbial growth in the broth. The minimum detection limit was calculated as 1.3

Log<sub>10</sub> CFU/cm<sup>2</sup> and was subtracted from the initial log concentration number when samples resulted in zero colony plate counts with a positive enrichment.

## Incubation and Grow-Out of Hatching Eggs

After PUV light treatment, fertile eggs were placed in a commercial incubator (Chick Master, Englewood, NJ) and incubated for 18 d. From the set day through day 18 of incubation, eggs were rotated 45° every hour at 37.6°C and 47.5% relative humidity. From day 18 until hatch, eggs were incubated in a commercial hatcher (Chick Master) maintained at 36.8°C and 65% relative humidity. Percent fertility was determined manually using an egg candler to observe embryonic development after 5 d of incubation. After 21 d of incubation, the percent hatch and hatchability were measured and recorded as a percentage of chicks from all set eggs and the percentage of chicks from the fertile eggs set, respectively. Hatched treatment chicks were randomly distributed to pullet rearing cages with 25 chicks per cage and 20 replicate cages per treatment. Chicks were fed a commercial pullet starter crumble diet. Average bird weight was calculated by dividing cage weight by the number of birds in each respective cage. Weights were recorded immediately after hatch and at day 7 and day 42 of rearing. Additionally, percent livability was calculated at 7 and 42 d of rearing as a percentage of chicks placed. The procedures followed during this study and the training of the personnel were approved by The Pennsylvania State University Institutional Animal Care and Use Committee, IACUC #01193.

## Statistical Analysis

Data analysis was performed using the Statistical Analysis Software 9.4 (SAS, Cary, NC). Table egg parameters were evaluated using a 1-way ANOVA (PROC GLM) for the effect of PUV light on microbial reduction and change in surface temperature with 15 measurements per treatment. Fertile eggs were evaluated for percent fertility, hatch, hatchability, and livability at 7 and 42 d using PROC GLM with an ARC SIN transformation of the percentage data. PROC GLIMIX was used to evaluate average bird weight at 1, 7, and 42 d of age. Tukey's multiple comparison test was used to separate means when the F-test was significant,  $P \leq 0.05$  (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### Microbial Response after PUV Light Treatment

The Log<sub>10</sub> reduction of *E. coli* K12-NSR and *E. faecium* on the surface of shell eggs was evaluated after PUV light treatment. For eggs inoculated with *E. coli* K12-NSR, there was a significant reduction after exposure to PUV light ( $P < 0.05$ ). Microbial reduction was quantified by comparing PUV-treated samples to untreated (control)

**Table 1.** Microbial reduction on the surface of table eggs after treatment by PUV light ( $\pm$ SD).

Microorganism	Energy(J/cm <sup>2</sup> )	Reduction(Log <sub>10</sub> CFU/cm <sup>2</sup> )	CFU observed(% no-growth) <sup>1</sup>	Selective enrichment(% positive) <sup>2</sup>	General enrichment(% positive) <sup>3</sup>
<i>Escherichia coli</i> K12-NSR	1.0	$\geq 3.43 \pm 0.55^b$	60%	93%	100%
	2.4	$\geq 4.00 \pm 0.79^{ab}$	73%	53%	60%
	3.1	$\geq 3.76 \pm 0.61^b$	80%	53%	60%
	4.9	$\geq 4.54 \pm 0.38^a$	100%	0%	20%
	SEM	0.156			
P-value	$P < 0.0001$				
<i>Enterococcus faecium</i>	1.0	$2.03 \pm 0.32^c$	0%	n/a	100%
	2.4	$2.81 \pm 0.63^b$	0%	n/a	100%
	3.1	$2.98 \pm 0.64^b$	13%	n/a	100%
	4.9	$3.52 \pm 0.47^a$	13%	n/a	100%
	SEM	0.137			
P-value	$P < 0.0001$				

<sup>a-c</sup>Means within microorganism without common superscripts are significantly different ( $P < 0.05$ ).

Abbreviations: PUV, pulsed ultraviolet; TSBYE-NS, tryptic soy broth supplemented with 0.6% of yeast extract and 100 mg/mL of both nalidixic acid and streptomycin sulfate

<sup>1</sup>Percent of agar plates (n = 15) that did not result in growth of colony forming units.

<sup>2</sup>TSBYE-NS for selective enrichment of *E. coli* K12-NSR.

<sup>3</sup>TSBYE for general enrichment for *E. coli* and *E. faecium*.

samples. Microbial reduction was  $\geq 3.43$ ,  $\geq 4.00$ ,  $\geq 3.76$ , and  $\geq 4.54$  Log<sub>10</sub> CFU/cm<sup>2</sup> after 1.0, 2.4, 3.1, or 4.9 J/cm<sup>2</sup> treatment, respectively (Table 1). At each treatment level, at least 1 egg resulted in positive growth on selective agar. Therefore, for samples that did not produce any colonies, but had a positive enrichment, a final microbial reduction was calculated by subtracting the minimum detection limit (1.3 Log<sub>10</sub> CFU/cm<sup>2</sup>) from the initial log concentration. After 4.9 J/cm<sup>2</sup> of PUV light exposure, there was complete surface inactivation of *E. coli* K12-NSR with no growth in selective enrichment. Reduction of *E. coli* K12-NSR observed on the surface of eggs after a total energy of 4.9 J/cm<sup>2</sup> was significantly greater compared with eggs treated with 1.0 and 3.1 J/cm<sup>2</sup> ( $P < 0.05$ ). There was no significant difference in reduction of *E. coli* K12-NSR for eggs treated with 1.0, 2.4, and 3.1 J/cm<sup>2</sup> ( $P > 0.05$ ).

For eggs inoculated with *E. faecium*, there was significant reduction after exposure to PUV light ( $P < 0.05$ ). Microbial reduction observed was 2.03, 2.81, 2.98, and 3.52 Log<sub>10</sub> CFU/cm<sup>2</sup> after 1.0, 2.4, 3.1, or 4.9 J/cm<sup>2</sup> treatment, respectively (Table 1). Reduction of *E. faecium* observed on the surface of eggs after a total energy of 4.9 J/cm<sup>2</sup> was significantly greater compared with eggs treated with 1.0, 2.4, and 3.1 J/cm<sup>2</sup> ( $P < 0.05$ ). There was no significant difference in reduction of *E. coli* K12-NSR for eggs treated with 2.4 and 3.1 J/cm<sup>2</sup> ( $P > 0.05$ ).

**Table 2.** Temperature change on the surface of table eggs after PUV light treatment ( $\pm$ SD).

Treatment (J/cm <sup>2</sup> )	Temperature ( $\Delta^\circ$ C)
1.0	$0.24 \pm 0.15^c$
2.4	$0.26 \pm 0.18^c$
3.1	$0.61 \pm 0.32^b$
4.9	$1.20 \pm 0.29^a$
SEM	0.067
P-value	$< 0.0001$

<sup>a-c</sup>Means without common superscripts are significantly different ( $P < 0.05$ ).

Abbreviations: PUV, pulsed ultraviolet.

Though evaluated separately, *E. coli* K12-NSR and *E. faecium* appear to have differing germicidal responses after exposure to PUV light at equal energy when initial inoculation concentrations were similar. For eggs inoculated with *E. coli* K12-NSR, every treatment level had agar plates with no colony growth. At 1.0, 2.4, 3.1, and 4.9 J/cm<sup>2</sup> of total energy, 60, 73, 80, and 100% of TSBYE-NS plates did not result in the growth of *E. coli* colonies. In comparison, *E. faecium* resulted in colony growth on m-Enterococcus agar at all treatment levels. At 4.9 J/cm<sup>2</sup> of total energy, *E. coli* K12-NSR resulted in a reduction of  $\geq 4.54$  Log<sub>10</sub> CFU/cm<sup>2</sup> compared with 3.52 Log<sub>10</sub> CFU/cm<sup>2</sup> for *E. faecium*. The difference in observed germicidal response is likely associated with the physiological differences between the microorganisms. *E. coli* K12-NSR are gram-negative and rod shaped, whereas *E. faecium* are gram-positive and cocci shaped. The thicker layer of cell wall peptidoglycan associated with gram-positive bacteria may be contributing a protective benefit against the effects of PUV light exposure. Koutchma (2009) reported that gram-positive bacteria are more resistant to conventional UV light than gram-negative bacteria. Rowan et al. (1999) and Anderson et al. (2000) both concluded that susceptibility of microorganisms to PUV light was greater for gram-negative bacteria compared with gram-positive bacteria.

Regardless of germicidal response differences observed between *E. coli* K12-NSR and *E. faecium*, reductions from this study are similar to previous shell egg decontamination research with PUV light. Keklik et al. (2010) reported between 1.3 to 5.3 Log<sub>10</sub> CFU/cm<sup>2</sup> reduction of *Salmonella* Enteritidis on 2.0 cm<sup>2</sup> portions of eggshells exposed to 1.2 to 24.8 J/cm<sup>2</sup> of PUV light, without any visible damage to the surface of the egg. It was reported that greater exposures, 24.8 to 35.3 J/cm<sup>2</sup>, resulted in surface temperature increases of 13.3°C to 16.3°C. Hierro et al. (2009) and Lasagabaster et al. (2011) inoculated the entire surface of shell eggs with *Salmonella* Enteritidis and *Salmonella* Typhimurium,

**Table 3.** Temperature change on the surface of hatching eggs after PUV light treatment ( $\pm$ SD).

Treatment	Temperature ( $\Delta^\circ\text{C}$ )
4.9 J/cm <sup>2</sup>	1.3 $\pm$ 0.09 <sup>c</sup>
24.4 J/cm <sup>2</sup>	4.4 $\pm$ 2.0 <sup>b</sup>
48.8 J/cm <sup>2</sup>	7.8 $\pm$ 1.9 <sup>a</sup>
SEM	0.339
P-value	<0.001

<sup>a-c</sup>Means without a common superscript are significantly different ( $P < 0.05$ ).

Abbreviation: PUV, pulsed ultraviolet.

respectively, before treatment using a benchtop PUV light system. In these studies, PUV flashlamps were positioned above and below a single egg held stationary in a fixed position. After 12 J/cm<sup>2</sup> of PUV exposure, [Hierro et al. \(2009\)](#) reported that the reduction of *Salmonella* Enteritidis on unwashed eggs was 2.49 Log<sub>10</sub> CFU/cm<sup>2</sup>, with 80% of the samples achieving the maximum observable decontamination of 3.6 Log<sub>10</sub> CFU/cm<sup>2</sup>. At 2.1 J/cm<sup>2</sup> energy of PUV light, [Lasagabaster et al. \(2011\)](#) reported a 4.9 Log<sub>10</sub> CFU/cm<sup>2</sup> reduction of *S. Typhimurium* on unwashed eggs. The findings of the current study are mostly consistent with the reports cited above, but further work directly comparing benchtop and conveyor PUV light systems may be illuminating.

### Energy and Temperature Profiles During Pulsed UV Treatment

Owing to the broad-spectrum energy delivered by the PUV lamp, surface temperature of eggs was measured immediately following treatment. Treatment by PUV light produced a significant ( $P < 0.05$ ) increase in the surface temperature of the eggs. The initial surface temperature of the eggs was  $21.5 \pm 1.1^\circ\text{C}$ . After 1.0, 2.4, 3.1, and 4.9 J/cm<sup>2</sup>, surface temperature increased  $0.24^\circ\text{C}$ ,  $0.26^\circ\text{C}$ ,  $0.61^\circ\text{C}$ , and  $1.20^\circ\text{C}$ , respectively ([Table 2](#)). In another study, the change in temperature on the surface of eggs treated with a total energy of 2.1 to 10.5 J/cm<sup>2</sup> was reported to increase  $1.2^\circ\text{C}$  to  $4.2^\circ\text{C}$ , respectively ([Lasagabaster et al., 2011](#)). The apparent difference in egg surface temperatures between previous and current studies may be because of thermodynamic differences in the PUV light systems. Using an enclosed PUV light benchtop system, such as the one used by

[Lasagabaster et al. \(2011\)](#), the opportunity for heat to build up in the treatment space is much greater compared with the open system evaluated in this study. In the current study, eggs are exposed to radiant energy on only one side as they rotate. The other, shaded side may be able to cool slightly while it is away from the illumination.

### Hatching Egg Response after PUV Light Treatment

Parameters of commercial hatchery performance including fertility, percent hatch, hatchability, and livability were evaluated after PUV light treatment of fertile eggs. The most favorable microbial reduction response was observed at 4.9 J/cm<sup>2</sup> for both *E. coli* K12 NSR and *E. faecium*; therefore, it was chosen as the minimum PUV light exposure level for treating hatching eggs. Additionally, in an effort to expose any adverse effects of PUV light treatment on fertile eggs, 24.4 and 48.8 J/cm<sup>2</sup> were evaluated as extreme treatment conditions.

Immediately after PUV light treatment of hatching eggs, the surface temperature was measured. As previously observed, increasing PUV light exposure significantly increased the egg surface temperature ( $P < 0.05$ ). The initial surface temperature was  $19.5^\circ\text{C} \pm 2.4^\circ\text{C}$  and increased 1.3, 4.4, and  $7.8^\circ\text{C}$  after exposure to a PUV light of 4.9, 24.4, and 48.8 J/cm<sup>2</sup>, respectively ([Table 3](#)). The greatest change in hatching egg surface temperature, observed at 48.8 J/cm<sup>2</sup>, resulted in a final temperature of  $29.4^\circ\text{C} \pm 2.4^\circ\text{C}$ . This final egg treatment temperature of  $29.4^\circ\text{C}$  is lower than commercial incubation temperatures set at approximately  $37.5^\circ\text{C}$ . These results indicate that at low levels of exposure, PUV light raises egg temperature to a degree that is not expected to affect hatching performance. Though, after prolonged exposure to PUV light, the rise in temperature may be undesirable.

There was no significant effect of PUV light on percent fertility, hatch, hatchability, or livability of fertile eggs exposed to 4.9, 24.4, and 48.8 J/cm<sup>2</sup> of PUV light ( $P > 0.05$ ; [Table 4](#)). Averaged across all treatments, fertility and hatch were 92.4 and 87.8%, respectively, whereas hatchability was 94.4%. However, there appeared to be a numerical difference in percent

**Table 4.** Percent fertility, hatchability, and livability of eggs that received PUV light treatment ( $\pm$ SD).

Treatment	% Fertility	% Hatchability <sup>1</sup>	% Hatch <sup>2</sup>	Livability %	
				1 wk	6 wk
Control	93.2 $\pm$ 1.5	97.0 $\pm$ 0.9	90.4 $\pm$ 2.1	99.3 $\pm$ 1.6	96.7 $\pm$ 5.6
4.9 J/cm <sup>2</sup>	92.0 $\pm$ 1.7	93.5 $\pm$ 2.2	86.0 $\pm$ 0.5	99.4 $\pm$ 2.0	98.0 $\pm$ 4.1
24.4 J/cm <sup>2</sup>	93.2 $\pm$ 1.9	93.3 $\pm$ 2.8	87.0 $\pm$ 3.8	99.2 $\pm$ 1.7	98.2 $\pm$ 2.4
48.8 J/cm <sup>2</sup>	93.2 $\pm$ 4.2	94.0 $\pm$ 1.9	87.6 $\pm$ 4.9	99.1 $\pm$ 2.0	98.1 $\pm$ 2.8
SEM	1.291	1.031	1.644	0.388	0.784
P-value	0.8834	0.086	0.3148	0.8473	0.4058

<sup>1</sup>Percent of fertile eggs.

<sup>2</sup>Percent of set eggs.



**Table 5.** Bird weight after hatch from eggs that received PUV light treatment ( $\pm$ SD).

Treatment	Average bird weight		
	Day 1	Day 7	Day 42
Control	39.44 $\pm$ 1.44	70.41 $\pm$ 3.01 <sup>a</sup>	477.09 $\pm$ 28.37
4.9 J/cm <sup>2</sup>	39.58 $\pm$ 0.92	65.43 $\pm$ 4.86 <sup>b</sup>	469.83 $\pm$ 20.49
24.4 J/cm <sup>2</sup>	39.94 $\pm$ 1.24	72.34 $\pm$ 4.95 <sup>a</sup>	469.47 $\pm$ 29.83
48.8 J/cm <sup>2</sup>	39.53 $\pm$ 0.89	69.40 $\pm$ 6.56 <sup>a</sup>	470.48 $\pm$ 25.51
SEM	0.255	1.124	5.873
<i>P</i> -value	0.5139	0.0005	0.7793

<sup>a-b</sup>Means without a common superscript are significantly different ( $P < 0.05$ ).

Abbreviation: PUV, pulsed ultraviolet.

hatchability between the untreated and treated eggs ( $P = 0.086$ ). Untreated eggs resulted in a 90.4% hatchability, whereas treated eggs resulted in 86.0, 87.0, and 87.6% hatchability after 4.9, 24.4, and 48.8 J/cm<sup>2</sup> of PUV light exposure. After 1 and 6 wk, average chick livability was 99.3 and 98.5%, respectively. Pulsed UV light treatment did not contribute to teratogenic effects on developing embryos even at high treatment levels. The absence of teratogenic effects is similar to findings for fertile eggs exposed to continuous UV light by Scott (1993), for 1, 3, or 5 min of UV light before incubation with no adverse effects on embryonic development reported.

Average chick weights at hatch and after 7 and 42 d of age are reported in Table 5. There were no significant differences among treatment groups on the day of hatch and after 42 d of grow out ( $P > 0.05$ ). However, there were significant differences among treatment groups after 7 d of rearing ( $P < 0.05$ ). Eggs treated with 4.9 J/cm<sup>2</sup> of PUV light resulted in chicks that weighed significantly less compared with all other treatment groups ( $P < 0.05$ ). There is no obvious explanation for this observation, and this treatment difference did not continue into the 42-day body weight. Averaged across all treatment groups, the chicks weighed 39.6, 69.4, and 471.6 g on the day of hatch and after 7 and 42 d of grow-out, respectively.

In a study by Patterson et al. (1990), chlorine dioxide solution proved to be an effective decontamination intervention for hatching eggs, but extreme concentrations negatively affected both fertility and hatchability. Unlike the negative impacts of chlorine dioxide, PUV light appears to have no real negative effects on incubation or rearing parameters.

## CONCLUSIONS

At the minimum PUV light level evaluated (1.0 J/cm<sup>2</sup>), PUV light treatment achieved significant microbial reductions of  $\geq 3.43$  and 2.03 Log<sub>10</sub> CFU/cm<sup>2</sup> for *E. coli* K12-NSR and *E. faecium*, respectively, on the surface of shell eggs. At an extreme exposure level (48.8 J/cm<sup>2</sup>), PUV light treatment had no adverse effects on fertility, percent hatch, and livability of fertile eggs. Pulsed ultraviolet lights effect on hatchability was inconclusive and should be further evaluated. These

results indicate that PUV light is an effective antimicrobial treatment for all eggs, intended for consumption or commercial incubation. Further research is necessary to determine if interior egg characteristics are altered when PUV light is delivered to the exterior shell surface. Before commercial implementation, it would be beneficial to evaluate the effectiveness of PUV light using actual pathogenic strains that might be found on eggs.

## ACKNOWLEDGMENTS

This work was partially supported by the USDA National Institute of Food and Agriculture Federal Appropriations, Project PEN04696 and the Penn State College of Agricultural Sciences, Undergraduate Student Research Program. Also, the authors would like to thank Hy-Line North America (Elizabethtown, PA) for supplying the eggs used in this study and Xenon Corporation (Wilmington, MA) for the technical assistance with the PUV light technology.

## DISCLOSURES

The authors declare no conflicts of interest.

## REFERENCES

- Anderson, J. G., N. J. Rowan, S. J. MacGregor, R. A. Fouracre, and O. Farish. 2000. Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Trans. Plasma Sci.* 28:83–88.
- American Egg Board (AEB). 2019. Egg industry Overview. U.S. Egg Facts. Accessed June 2020. <https://www.aeb.org/farmers-and-marketers/industry-overview>.
- Boney, J. W., J. Jaczynski, J. L. Weidhaas, A. N. Bergeron, and J. S. Moritz. 2018. The effects of steam conditioning and antimicrobial inclusion on feed manufacturing and inactivation of *Enterococcus faecium*, a *Salmonella* surrogate. *J. Appl. Poult. Res.* 27:472–482.
- Cassar, J. R., E. W. Mills, J. A. Campbell, and A. Demirci. 2019. Pulsed UV light as a microbial reduction intervention for boneless/skinless chicken thigh meat. *Meat Muscle Biol.* 2:142.
- Cassar, J. R., B. Ouyang, K. Krishnamurthy, and A. Demirci. 2020. Microbial decontamination of food by light-based Technologies: ultraviolet (UV) light, pulsed UV light (PUV), and UV light-emitting Diodes (UV-LED). *Food safety Engineering*. Pages 493–521 in *Food Safety Engineering*. Springer, Cham, Gewerbestrasse, Switzerland.
- Catalano, C. R., and S. J. Knabel. 1994. Destruction of *Salmonella* Enteritidis by high pH and rapid chilling during simulated commercial egg processing. *J. Food Prot.* 57:592–595.
- Dewey-Mattia, D., K. Manikonda, A. J. Hall, M. E. Wise, and S. J. Crowe. 2018. Surveillance for foodborne Disease outbreaks - United States, 2009–2015. *MMWR Surveill. Summ.* 67:1–11.
- Hierro, E., S. Manzano, J. A. Ordóñez, L. de la Hoz, and M. Fernández. 2009. Inactivation of *Salmonella* enterica serovar Enteritidis on shell eggs by pulsed light technology. *Int. J. Food Microbiol.* 135:125–130.
- Keener, K. M. 2017. Shell egg pasteurization. Pages 165–175 in *Egg Innovations and Strategies for Improvements*. Academic Press, Elsevier, Cambridge, MA.
- Keklik, N. M., A. Demirci, P. H. Patterson, and V. M. Puri. 2010. Pulsed UV light inactivation of *Salmonella* Enteritidis on eggshells and its effects on egg quality. *J. Food Prot.* 73:1408–1415.
- Koutchma, T. 2009. Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food Bioproc. Technol.* 2:138–155.

- Kusstatscher, P., T. Cernava, S. Liebming, and G. Berg. 2017. Replacing conventional decontamination of hatching eggs with a natural defense strategy based on antimicrobial, volatile pyrazines. *Sci. Rep.* 7:1–8.
- Krishnamurthy, K., J. C. Tewari, J. Irudayaraj, and A. Demirci. 2010. Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. *Food Bioproc. Technol.* 3:93–104.
- Lasagabaster, A., J. C. Arboleya, and I. M. De Maranon. 2011. Pulsed light technology for surface decontamination of eggs: impact on Salmonella inactivation and egg quality. *Innovative Food Sci. Emerging Tech.* 12:124–128.
- Mellata, M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog. Dis.* 10:916–932.
- O'Bryan, C. A., S. C. Ricke, and P. G. Crandall. 2017. Chemical and Physical Sanitation and pasteurization methods for Intact shell eggs. Pages 373–390 in *Producing Safe Eggs*. Academic Press, Elsevier, Cambridge, MA.
- Ouyang, B., A. Demirci, and P. H. Patterson. 2020. Inactivation of *Escherichia coli* and Salmonella in liquid egg white by pulsed UV light and its effects on quality. *J. Food Process Eng.* 83:418–425.
- Patterson, P. H., S. C. Ricke, M. L. Sunde, and D. M. Schaefer. 1990. Hatching eggs sanitized with chlorine dioxide foam: egg hatchability and bactericidal properties. *Avian Diseases* 34:1–6.
- Rowan, N. J., S. J. MacGregor, J. G. Anderson, R. A. Fouracre, L. McIlvaney, and O. Farish. 1999. Pulsed-light inactivation of food-related microorganisms. *Appl. Environ. Microbiol.* 65:1312–1315.
- Scott, T. A. 1993. The effect of UV-light and air filtering system on embryo viability and microorganism load on the egg shell. *J. Appl. Poult. Res.* 2:19–25.
- Shane, S. M., and A. Faust. 1996. Evaluation of sanitizers for hatching eggs. *J. Appl. Poult. Res.* 5:134–138.
- Sonenshein, A. L. 2003. Killing of Bacillus Spores by High-Intensity Ultraviolet Light. Sterilization and Decontamination Using High Energy Light. Xenon Corporation, Wilmington, MA.
- Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill, New York, NY.
- Troscianko, J. 2014. A simple tool for calculating egg shape, volume and surface area from digital images. *Ibis* 156:874–878.
- Turtoi, M., and D. Borda. 2014. Decontamination of eggshells using ultraviolet light treatment. *Worlds Poult. Sci. J.* 70:265–278.
- United States Department of Agriculture USDA. 2000. United States standards, grades, and weight classes for shell eggs. AMS 56.210. Agriculture Marketing Service of the Poultry Programs of the USDA, UDSA, Washington, DC.
- United States Department of Agriculture USDA. 2020. National Agricultural Statistics Service. Chickens and Eggs. 2019 Summary. February 2020. ISSN:1948-9056. Washington, DC.
- Williams, J. E. 1970. Effect of high-level formaldehyde fumigation on bacterial populations on the surface of chicken hatching eggs. *Avian Dis.* 1:386–392.
- Vinayananda, C. O., N. Fairoze, C. B. Madhavaprasad, S. M. Byregowda, C. S. Nagaraj, P. Bagalkot, and N. Karabasanavar. 2017. Studies on occurrence, characterization, and decontamination of emerging pathogenic *Escherichia coli* (STEC, ETEC and EIEC) in table eggs. *Br. Poult. Sci.* 58:664–672.