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Evaluation of the effects of ultraviolet light on bacterial contaminants inoculated into whole milk and colostrum, and on colostrum immunoglobulin G

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Abstract

Raw milk and colostrum can harbor dangerous micro-organisms that can pose serious health risks for animals and humans. According to the USDA, more than 58% of calves in the United States are fed unpasteurized milk. The aim of this study was to evaluate the effect of UV light on reduction of bacteria in milk and colostrum, and on colostrum IgG. A pilot-scale UV light continuous (UVC) flow-through unit (45 J/cm²) was used to treat milk and colostrum. Colostrum and sterile whole milk were inoculated with *Listeria innocua*, *Mycobacterium smegmatis*, *Salmonella* serovar Typhimurium, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Acinetobacter baumannii* before being treated with UVC. During UVC treatment, samples were collected at 5 time points and bacteria were enumerated using selective media. The effect of UVC on IgG was evaluated using raw colostrum from a nearby dairy farm without the addition of bacteria. For each colostrum batch, samples were collected at several different time points and IgG was measured using ELISA. The UVC treatment of milk resulted in a significant final count (log cfu/mL) reduction of *Listeria monocytogenes* (3.2 ± 0.3 log cfu/mL reduction), *Salmonella* spp. (3.7 ± 0.2 log cfu/mL reduction), *Escherichia coli* (2.8 ± 0.2 log cfu/mL reduction), *Staph. aureus* (3.4 ± 0.3 log cfu/mL reduction), *Streptococcus* spp. (3.4 ± 0.4 log cfu/mL reduction), and *A. baumannii* (2.8 ± 0.2 log cfu/mL reduction). The UVC treatment of milk did not result in a significant final count (log cfu/mL) reduction for *M. smegmatis* (1.8 ± 0.5 log cfu/mL reduction). The UVC treatment of colostrum was significantly associated with a final reduction of bacterial count (log cfu/mL) of *Listeria* spp. (1.4 ± 0.3 log cfu/mL reduction), *Salmonella* spp. (1.0 ± 0.2 log cfu/mL reduction), and *Acinetobacter* spp. (1.1 ± 0.3 log cfu/mL reduction), but not of *E. coli* (0.5 ± 0.3 log cfu/mL reduction), *Strep. agalactiae* (0.8 ± 0.2 log cfu/mL reduction), and *Staph. aureus* (0.4 ± 0.2 log cfu/mL reduction). The UVC treatment of colostrum significantly decreased the IgG concentration, with an observed final mean IgG reduction of approximately 50%. Development of new methods to reduce bacterial contaminants in colostrum must take into consideration the barriers imposed by its opacity and organic components, and account for the incidental damage to IgG caused by manipulating colostrum.

Keywords

ultraviolet light; colostrum; immuno-globulin G; milk

INTRODUCTION

Feeding preweaned calves raw milk and colostrum has the potential to increase calf morbidity and mortality due to ingestion of pathogenic agents. Some important pathogenic bacteria identified in raw milk and colostrum include *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Staphylococcus aureus*, and *Streptococcus* spp. (Elizondo-Salazar and Heinrichs, 2009; Oikonomou et al., 2012; Pearce et al., 2012).

Pasteurization is commonly used on dairy farms as an effective preventive method to reduce bacterial load in the milk fed to calves. However, pasteurization is an energy-demanding process with high capital and operating costs (Krishnamurthy et al., 2004). Heat treatment of colostrum at a high temperature for a short time has been associated with a decrease in IgG concentration of 22 to 27% (Stabel et al., 2004). However, heat treatment of colostrum at a lower temperature (60°C) for 60 min has been observed to have no significant changes in the IgG concentration compared with raw colostrum and has been suggested as a viable option for treatment of colostrum at the dairy farm (Johnson et al., 2007; Donahue et al., 2012).

The classical use of UV light has occurred in biological safety cabinets in laboratories, although in recent years its use has been extended to inactivation of microorganisms in the food-processing industry, in potable water, and in wastewater (Gómez et al., 2011). Ultraviolet light inactivates microorganisms by forming pyrimidine dimers in RNA and DNA, which can interfere with transcription and replication (Goosen and Moolenaar, 2008; Cutler and Zimmerman, 2011). The germicidal effect of UV light treatment is dependent on microbial exposure, but when used on opaque foods with irregular surfaces, UV light may cause less microbial destruction. Although the opacity and high absorption coefficient of milk has been considered a barrier to the use of UV light as a disinfectant, UV light treatment of milk has been shown to reduce bacterial counts of *L. monocytogenes* in goat milk and *Staph. aureus* in cow milk (Matak et al., 2005).

Therefore, the objectives of this study were to determine the effect of UV light treatment on the count (log cfu/mL) reduction of bacteria (*Listeria innocua*, *Mycobacterium smegmatis*, *Salmonella* serovar Typhimurium, *E. coli*, *Staph. aureus*, *Streptococcus agalactiae*, and *Acinetobacter baumannii*) inoculated into sterile milk and colostrum, and on IgG concentration in colostrum.

MATERIALS AND METHODS

Bacteria for Inoculation of Milk and Colostrum

Seven bacterial species of concern to animal and human health were selected as inoculants in the milk trials: *L. innocua*, *M. smegmatis*, *Salmonella* serovar Typhimurium, *E. coli*, *Staph. aureus*, *Strep. agalactiae*, and *A. baumannii*. For *Salmonella* spp., *E. coli*, *Staph.*

aureus, *Strep. agalactiae*, and *A. baumannii*, 2 to 3 different strains were selected for inoculation of milk (Table 1). For the remaining bacterial species, only 1 strain was selected for inoculation of milk. *Listeria innocua* and *M. smegmatis* were chosen as surrogates for *L. monocytogenes* and MAP, respectively (Bannantine et al., 1997; Friedly et al., 2008). For the colostrum experiments, only 1 strain of *L. innocua* [American Type Culture Collection (ATCC) 33090], *Salmonella* serovar Typhimurium (ATCC 14028), *E. coli* (ATCC 25922), *Staph. aureus* (ATCC 27708), *Strep. agalactiae* (SAG 2), and *A. baumannii* (ATCC 19606) were chosen as inoculants (Table 1).

Preparation of Cell Suspension

Each bacterial strain was cultured individually on growth medium specific for the species, following the manufacturers' instructions (Table 2). Briefly, bacterial stocks stored at -80°C were transferred using an inoculation loop into broth growth medium and grown under conditions ideal for each bacterial species, as described in Table 2. After incubation, a bacterial pellet was harvested from the broth growth medium by centrifugation ($4,000 \times g$ for 22 min) and resuspended in broth medium to create bacteria stocks by pipetting 800 μL of the solution into Eppendorf tubes containing 200 μL of 80% glycerol. Bacterial stocks were stored at -80°C until used for inoculation of milk and colostrum (Perez et al., 2003; Altic et al., 2007; Hegde et al., 2007; Choi et al., 2008; Nicolaou et al., 2011).

Pilot-Scale UV Light Machine

The experimental device tested consisted of a pilot-scale UV light continuous (UVC) flow-through unit (GEA Farm Technologies, Naperville, IL), with turbulators made of stainless steel, a pump unit for fluids (flow rate of 65 l/min; 230-V pump with 20-A breaker), and 1 UV lamp module (45 J/cm^2 ; dimensions: 610 mm wide, 1,665 mm long, and 500 mm deep). The UV lamp was inserted into a transparent quartz tube to allow exposure of the milk to the UV light (Figure 1). The time necessary for a batch of 4 L of milk or colostrum to fully flow through the UVC unit was 2 min and 30 s.

Sample Preparation and Inoculation

Commercial pasteurized-homogenized whole milk (3.25% fat content) was purchased at a local supermarket and autoclaved (121°C for 32 min) before inoculation with bacteria. Two different bacteria-inoculated milk batches were used, and their bacterial composition and mean concentration (log cfu/mL) in the milk before initializing the UV light treatment were (1) milk inoculated with only *M. smegmatis* ($4.7 \pm 0.5 \text{ log cfu/mL}$) and (2) milk inoculated with a bacterial cocktail made from combining 1 strain of *L. innocua* ($7.6 \pm 0.2 \text{ log cfu/mL}$), *Salmonella* serovar Typhimurium ($7.4 \pm 0.2 \text{ log cfu/mL}$), *E. coli* ($6.2 \pm 0.2 \text{ log cfu/mL}$), *Staph. aureus* ($7.3 \pm 0.2 \text{ log cfu/mL}$), *Strep. agalactiae* ($5.2 \pm 0.3 \text{ log cfu/mL}$), and *A. baumannii* ($6.4 \pm 0.2 \text{ log cfu/mL}$). *Mycobacterium smegmatis* was inoculated in a separate batch to increase the precision of the enumeration on the selective growth media. For *Salmonella* spp., *E. coli*, *Staph. aureus*, *Strep. agalactiae*, and *A. baumannii*, 2 to 3 different strains were selected for inoculation of milk, and to compare the effects of UVC on each individual strain, sterile milk batches were inoculated with only 1 bacterial strain belonging

to the same bacterial species at a time. Four repetitions were performed for each bacterial strain and each bath contained 4 L of milk.

Raw colostrum pooled from multiple cows was obtained from a nearby dairy farm. Colostrum used in the study was not autoclaved. Raw colostrum used to evaluate the effect of UVC on bacterial contaminants was inoculated with a bacterial cocktail composed of 1 bacterial strain. The bacterial strains selected and the mean concentration (log cfu/mL) in the colostrum for these bacteria species before initializing the UV light treatment were *Listeria* spp. (strain ATCC 33090; 4.6 ± 0.2 log cfu/mL), *Salmonella* spp. (strain ATCC 14028; 7.6 ± 0.2 log cfu/mL), *E. coli* (strain ATCC 25922; 6.7 ± 0.3 log cfu/mL), *Staph. aureus* (strain ATCC 27708; 7.8 ± 0.1 log cfu/mL), *Strep. agalactiae* (strain SAG 2; 3.7 ± 0.2 log cfu/mL), and *Acinetobacter* spp. (strain ATCC 19606; 5.0 ± 0.2 log cfu/mL; (Table 1). Four repetitions were performed for each bacterial species and each bath contained 4 L of colostrum.

Testing the Effect of UVC on Bacterial Counts

To evaluate the ability of UVC treatment to reduce bacterial counts in milk and colostrum, samples were collected at 5 time points: (1) UV lamp and pump both off and after mixing the milk or colostrum manually with a sterile rod for 1 min (**OFF**), (2) UV lamp off and after the pump had been on for 2 min and 30 s (40 cycles; **PT**), (3) UV lamp on and after the pump had been on for 2 min and 30 s (40 cycles; **T1**), (4) UV lamp on and after the pump had been on for 5 min (80 cycles; **T2**), and (5) UV lamp on and after the pump had been on for 7 min and 30 s (120 cycles; **T3**; Figure 2). Samples OFF and PT were collected before any exposure to UV light with the aim of evaluating the effect of the pump alone on bacterial contaminants. When collecting UV light-exposed samples (T1, T2, and T3), the UV lamp was switched on for 5 min before switching on the pump, allowing the UV lamp to achieve an optimal activity level.

UVC Unit Cleaning Protocol

Between milk or colostrum batches, a 5-step cleaning protocol was implemented for the UVC unit as follows: (1) rinsed with 6 L of warm water and sodium hypo-chlorite (The Clorox Co., Oakland, CA) for 5 min, (2) rinsed with 4 L of warm water and an alkaline detergent (TRI-PFAN; GEA Farm Technologies) for 5 min, (3) rinsed with 4 L of warm water and a low-foam acid cleaner (LAC; GEA Farm Technologies) for 3 min, (4) rinsed with 8 L of warm water and sodium hypochlorite (The Clorox Co.) for 3 min, and (5) rinsed with 4 L of hot water ($>75^{\circ}\text{C}$) for 3 min.

Microbiology Assays

Both milk and colostrum samples were serially diluted (10^{-1} to 10^{-8}) and 20- μL aliquots were plated and incubated aerobically using selective media and conditions specific for each bacterial species (Table 2). An estimate of the colony-forming units per milliliter was made by calculating the average number of colonies (from triplicate determinations) and multiplying this number by the reciprocal of the dilution factor.

Testing the Effect of UVC on Colostrum IgG

Raw colostrum pooled from multiple cows was obtained from a nearby dairy farm. Colostrum used in the study was not autoclaved. To evaluate the effect of UVC on colostrum IgG, samples were collected at 7 time points: (1) UV lamp and pump both off and after mixing the colostrum manually for 1 min (OFF), (2) UV lamp off and after the pump had been on for 2 min and 30 s (40 cycles; PT), (3) UV lamp on and after the pump had been on for 2 min and 30 s (40 cycles; T1), (4) UV lamp on and after the pump had been on for 5 min (80 cycles; T2), (5) UV lamp on and after the pump had been on for 7 min and 30 s (120 cycles; T3), (6) UV lamp on and after the pump had been on for 10 min (160 cycles; T4), and (7) UV lamp on and after the pump had been on for 12 min and 30 s (200 cycles; T5). When collecting UV light-exposed samples (T1, T2, T3, T4, and T5), the UV lamp was switched on for 5 min before switching on the pump, allowing the UV lamp to achieve an optimal activity level. Because of the limited research on the effects of treatment of colostrum with UV light, a longer UVC exposure time of colostrum (up to T5 instead of only T3) was used, with the objective of obtaining additional information on the cumulative incidental effects of UV light on colostrum IgG. In total, 9 repetitions were performed and each bath contained 4 L of colostrum. Immunoglobulin G levels were quantified using ELISA (Immuno-Tek Bovine IgG ELISA Kit; ZeptoMetrix Corp., Buffalo, NY).

Statistical Analysis

Microbial counts (cfu/mL) were converted into logarithmic units for statistical analysis. The effect on milk and colostrum of flowing through the UVC pump in the absence of UV irradiation was tested by observing the variance of bacterial count (log cfu/mL) from samples at OFF and PT by using ANOVA in the statistical software SAS (SAS Institute Inc., Cary, NC).

To evaluate the effect of UV light treatment on bacterial species added to sterile milk and to colostrum, for each bacteria species, a mixed linear model accounting for repeated measures (batches) was used (PROC MIXED; SAS Institute Inc.). In this model, the dependent variable was the count (log cfu/mL) for the bacterial species and the independent variable was the sampling time points (PT, T1, T2, and T3). For bacteria species where more than 1 bacterial strain was used, an explanatory variable accounting for each individual bacterial strains and its interaction with sampling time points (PT, T1, T2, and T3) was added and retained in the model if the interaction was significant. A Tukey honestly significant difference (HSD) test was performed in SAS to identify pairs of sampling time points that had significantly different mean count (log cfu/mL) of each bacterial species tested.

The PROC REG procedure in SAS was used to complete a simple linear regression to evaluate the effect of UVC on colostrum IgG. IgG measured in colostrum was offered to the model as the dependent variable and the 7 sampling time points (OFF, MIX, T1, T2, T3, T4 and T5) were offered as independent variables. To illustrate the linear association of colostrum IgG and treatment by UVC, a simple linear scatter plot was computed using MedCalc Version 12.4.0.0 (MedCalc Software, Mariakerke, Belgium). All statistical models, variables and their interactions were considered significant when their respective *P*-values were <0.05.

RESULTS

Effect of UVC on Bacterial Contaminants in Milk Samples

The UVC treatment of milk resulted in a significant count (log cfu/mL) reduction at T3 for *L. monocytogenes* (3.2 ± 0.35 log cfu/mL reduction), *Salmonella* spp. (3.7 ± 0.27 log cfu/mL reduction), *E. coli* (2.8 ± 0.22 log cfu/mL reduction), *Staph. aureus* (3.4 ± 0.28 log cfu/mL reduction), *Streptococcus* spp. (3.4 ± 0.45 log cfu/mL reduction), and *A. baumannii* (2.8 ± 0.24 log cfu/mL reduction). However, UVC treatment of milk did not result in a significant count (log cfu/mL) reduction at T3 for *M. smegmatis* (1.8 ± 0.5 log cfu/mL reduction; $P = 0.07$; Figure 3). No significant difference was observed in the bacterial count (log cfu/mL) from OFF to PT for all milk trials (UV light was switched off during these 2 sampling points). No significant difference in the bacterial count (log cfu/mL) at different time points was observed at the bacterial species level between strains of *Salmonella* spp., *E. coli*, *Staph. aureus*, *Strep. agalactiae*, and *A. baumannii*, for which more than 1 strain per bacterial species was used to inoculate distinct sterile milk batches.

Effect of UVC on Bacterial Contaminants and IgG in Colostrum Samples

Although increasing the number of cycles that the colostrum was exposed to UVC radiation lowered the count (log cfu/mL) for all 6 bacterial species tested, the UVC treatment of colostrum was significantly associated with a count (log cfu/mL) reduction at T3 of *Listeria* spp. (1.4 ± 0.3 log cfu/mL reduction), *Salmonella* spp. (1.0 ± 0.2 log cfu/mL reduction), and *Acinetobacter* spp. (1.1 ± 0.3 log cfu/mL reduction), but not of *E. coli* (0.5 ± 0.3 log cfu/mL reduction), *Strep. agalactiae* (0.8 ± 0.2 log cfu/mL reduction), and *Staph. aureus* (0.4 ± 0.2 log cfu/mL reduction; Figure 4). No significant difference was observed in the log cfu/ml for any of the 6 bacterial species tested from OFF to PT.

Treatment of colostrum with UVC had a negative linear association with IgG concentration in colostrum ($P = 0.019$; Figure 5). A mean IgG reduction of approximately 50% was observed from OFF (70.5 g/L; 95% CI = 51–90 g/L) to T5 (35.1 g/L; 95% CI = 15–54 g/L).

DISCUSSION

With the exception of *M. smegmatis*, a significant reduction in the count (log cfu/mL) for all bacterial species tested was observed as the milk was treated with the UVC. The UVC used in the trials had the same UV light (45 J/cm^2) and followed the similar dosage protocol recommended for the UV Pure system (GEA Farm Technologies), a commercially available machine to treat milk using UV light. Similar to our findings, a field trial by Teixeira et al. (2013) using the UV Pure system to treat hospital milk from 1 dairy observed significant reductions in bacterial counts, with logarithmic reductions in bacterial colony counts for total bacterial count, *E. coli*, *Staph. aureus*, and *Streptococcus* spp. of 3.3, 1.7, 0.2, and 2.0, respectively. The same study also evaluated the pasteurization of hospital milk (72°C for 15 s) and observed logarithmic reductions in bacterial colony counts for total bacterial count, *E. coli*, *Staph. aureus*, and *Streptococcus* spp. of 5.2, 1.2, 0.2, and 2.4, respectively (Teixeira et al., 2013). Teixeira et al. (2013) concluded that overall heat treatment of hospital milk was more effective than UV treatment in decreasing bacterial counts. A study by Matak et al.

(2005) using a machine with 8 UV lamps (254 nm and 90% emittance) observed a significant 5-log reduction of *L. monocytogenes* in UV-treated goat milk.

The lack of an effect of UV light on reduction of mycobacteria in milk identified in our study was also observed by Donaghy et al. (2009) when using a pilot-scale UV light machine (30-W UVC output) to treat milk inoculated with MAP. They concluded that the apparent resistance of MAP to UV light treatment could have been caused by the opacity of milk and the recalcitrance of MAP to inimical treatments. Because MAP is a slow-growing mycobacterium which can have an incubation period for enumeration of colonies by solid culture of more than 3 mo, *M. Smegmatis* was chosen to be used as a surrogate in the current study (Grant et al., 2001; Kralik et al., 2012). *Mycobacterium smegmatis* is a fast-growing species of mycobacteria with no linkage to human disease, having dormancy genes similar to those in MAP (Whittington et al., 2004). *Mycobacterium smegmatis* has often been used as a surrogate for pathogenic bacteria such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and MAP (Bannantine et al., 1997; Dhandayuthapani et al., 1997; Harris and Barletta, 2001; Chaturvedi et al., 2007).

In this study, UVC treatment of colostrum caused a significant reduction in the bacterial count (log cfu/mL) for *Listeria* spp., *Salmonella* spp., and *Acinetobacter* spp., but not of the remaining bacteria species. A study by Teixeira et al. (2013) observed an average logarithmic reduction in bacterial reduction in colostrum treated with a UV Pure system of 1.7, 2.2, 0.4, and 2.5 for the total bacterial count, *E. coli*, *Staph. aureus*, and *Streptococcus* spp., respectively. When using heat treatment of colostrum at 63°C for 60 min in the same study, they observed an average logarithmic reduction in bacterial reduction in colostrum of 3.5, 4.5, 0.4, and 2.5 for the total bacterial count, *E. coli*, *Staph. aureus*, and *Streptococcus* spp., respectively (Teixeira et al., 2013). Teixeira et al. (2013) concluded that the overall heat treatment of colostrum was more effective than UV treatment in decreasing bacterial counts. Additional research using the same settings as used in our study is needed to allow a direct comparison of efficacy on reduction of bacterial counts when using UVC or other available approaches to treat milk or colostrum.

The lack of a significant bacterial reduction of some of the bacteria species after treatment of colostrum with UVC may be explained by a limited penetrating ability of UV light in colostrum, causing a lower reduction in bacterial counts. Liquids that have high light transmissivity can be effectively treated with UV light, but liquids with low light transmissivity, caused by particulate materials or organic compounds, may pose a barrier for UV treatment (Guerrero-Beltrán and Barbosa-Cánovas, 2004). A common measurement to assess the penetration of UV light through fluids is the absorption coefficient (AC), where less penetration of light occurs with higher AC. As an example, the AC for drinking water is around 0.02 to 0.1 (cm⁻¹), whereas the AC for milk is 300 (cm⁻¹), which illustrates a limitation of using UV light on opaque fluids (Guerrero-Beltrán and Barbosa-Cánovas, 2004). The challenges of using UV light to irradiate colostrum are even greater than those observed for milk, considering that colostrum has, on average, higher quantities of organic compounds such as fat (6.7% in colostrum vs. 4% in milk) and protein (14% in colostrum vs. 3.1% in milk; Godden, 2008).

Added to the potential reduction in exposure of bacteria to UV radiation in the colostrum, another additional explanation for the significant reduction in the bacterial count (log cfu/mL) for *Listeria* spp., *Salmonella* spp., and *Acinetobacter* spp. but not of the remaining bacteria species after treatment of colostrum samples with UVC could have been related to resistance of these bacteria species to UV radiation. Albarracín et al. (2012), who studied strains of *Acinetobacter* spp. isolated from ecosystems at high altitudes, observed strains resistant to high levels of UV radiation. The resistance of these strains was related in part to their ability to more effectively repair pyrimidine dimers compared with control strains. The mechanism of UV light inactivation of microorganisms is based on the formation of dimers in RNA and DNA, causing damage that can interfere with transcription and replication and, furthermore, cause bacterial death (Cutler and Zimmerman, 2011). Bacterial mechanisms to repair damage caused by UV light include direct reversal of the damage by a photolyase (photoreactivation), removal of the lesion by a DNA glycosylase, and nucleotide excision repair (Goosen and Moolenaar, 2008). Bacterial strains with more effective mechanisms of dimer repair could have lower count reductions in milk after exposure to UV light.

A negative linear relationship was observed between the duration of UVC treatment of colostrum and the concentration of IgG (Figure 5). A study by Teixeira et al. (2013) observed that colostrum treated with a commercial UV light flow-through machine caused a reduction in colostrum IgG of 42.2% compared with untreated colostrum. Although UVC was capable of significantly reducing the bacterial counts for *Listeria* spp., *Salmonella* spp., and *Strep. agalactiae* in colostrum, its use to treat colostrum fed to calves must be carefully considered and the potential loss of viable IgG taken into account. Colostrum IgG has been shown to be sensitive to pressure, heat, and acid, which affect the conformation of the immunoglobulin molecule and ultimately the immunological activity of the antibody (Hurley and Theil, 2011). Trujillo et al. (2007) observed that treatment of caprine colostrum at a pressure of 500 MPa caused a reduction in IgG of 40% compared with untreated colostrum. Heat treatment of colostrum has been shown to result in mean IgG losses as high as 24% after treatment at 63°C for 30 min (Meylan et al., 1996; Godden et al., 2003). However, recent studies have shown that heat treatment of colostrum at a slightly lower temperature (60°C) for 60 min had no significant changes in the IgG concentration compared with raw colostrum and furthermore decreased colostrum total plate counts ($-2.25 \log_{10}$) and coliform counts ($-2.49 \log_{10}$; Johnson et al., 2007; Donahue et al., 2012). Acidic pH has also been shown to cause denaturation and to reduce the antigen-binding activity of IgG in colostrum (Chen and Chang, 1998; Domínguez et al., 2001). Procedures that have the purpose of reducing the bacterial load present in colostrum must take into account the resulting effects on the immunological activity of the antibodies.

CONCLUSIONS

The UVC treatment of sterile, commercial whole milk inoculated with bacteria caused a significant reduction for all bacterial species tested except *M. smegmatis*. The UVC treatment of colostrum samples inoculated with bacterial contaminants resulted in a significant reduction in the bacterial count at T3 for *Listeria* spp., *Salmonella* spp., and *Acinetobacter* spp., but not of *E. coli*, *Strep. agalactiae*, and *Staph. aureus*. Moreover, UVC treatment of colostrum lowered the concentration of IgG in colostrum in relation to the

length of treatment duration. Processes that aim to reduce the bacterial count in colostrum must take into consideration the incidental effects on colostrum IgG.

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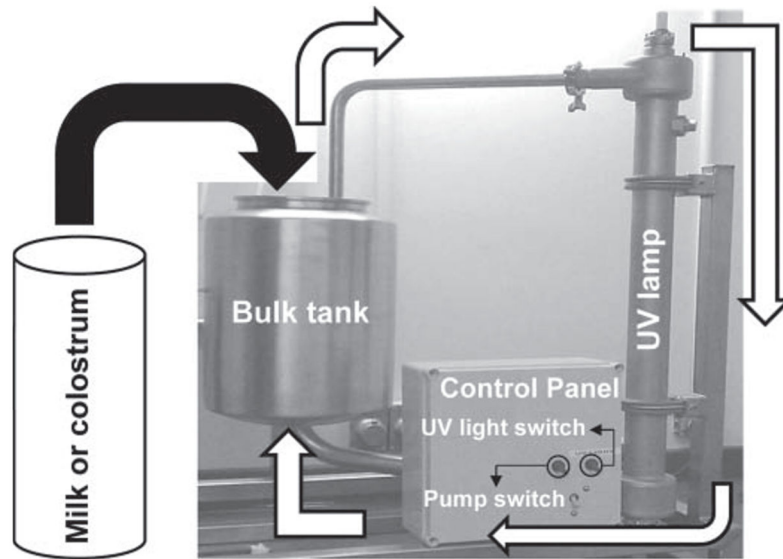


Figure 1. Flow-through UV light unit used for trials. White arrows indicate the continuous flow trajectory of fluids through the UV lamp and returning to the bulk tank when the pump was switched on.

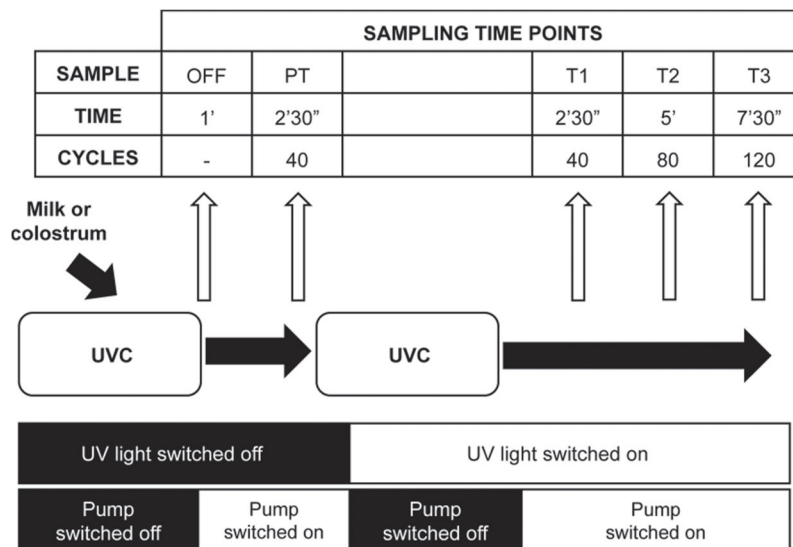


Figure 2. Ultraviolet light continuous (UVC) flow-through unit operation and description of exposure time and number of cycles the entire sample flowed through the UVC system before sample collection. To evaluate the ability of UVC treatment to reduce bacterial counts in milk and colostrum, samples were collected at 5 time points: (1) UV lamp and pump both off and after mixing the milk or colostrum manually with a sterile rod for 1 min (OFF), (2) UV lamp off and after the pump had been on for 2 min and 30 s (PT), (3) UV lamp on and after the pump had been on for 2 min and 30 s (T1), (4) UV lamp on and after the pump had been on for 5 min (T2), and (5) UV lamp on and after the pump had been on for 7 min and 30 s (T3).

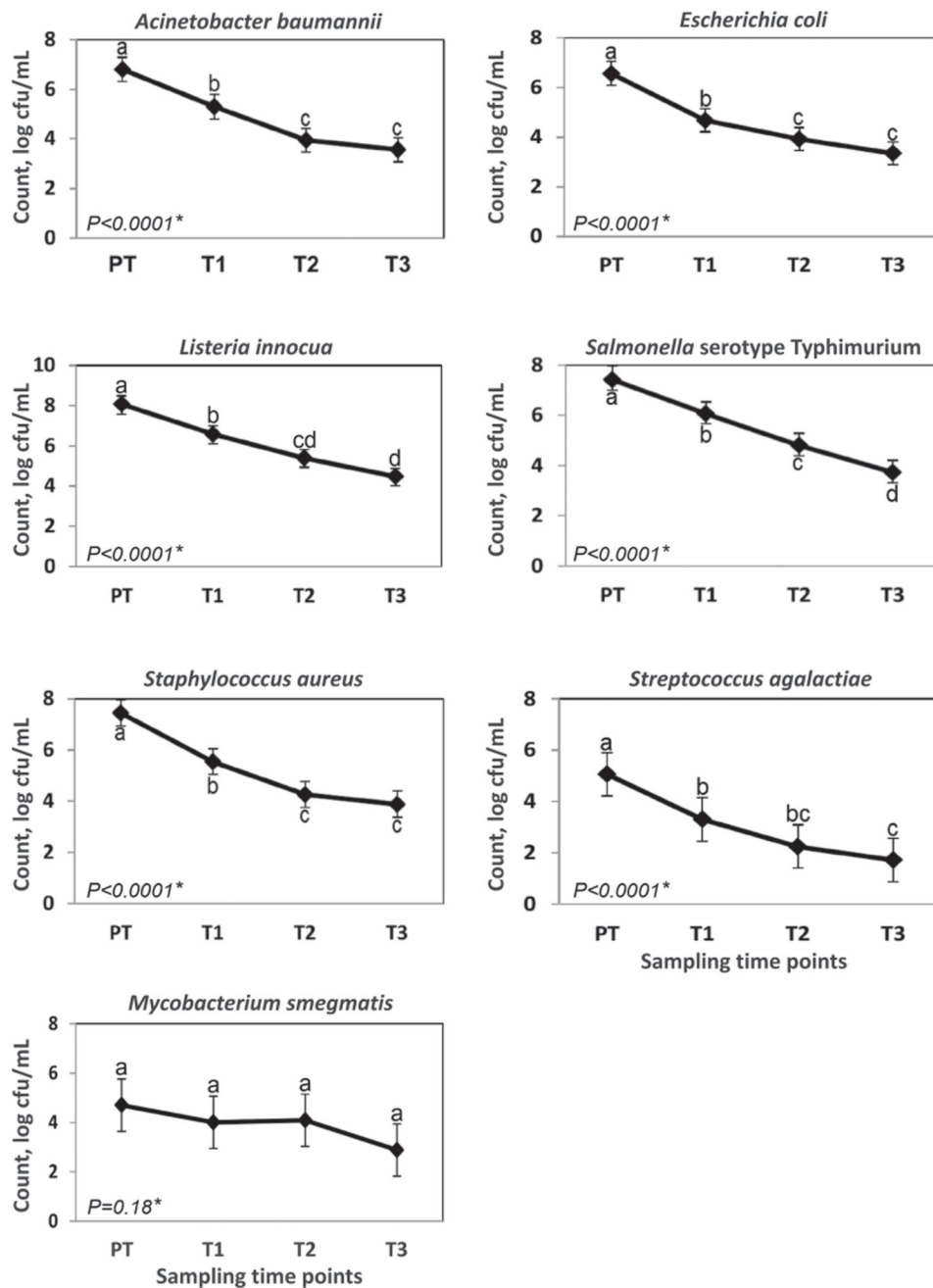


Figure 3. Counts (mean log cfu/mL) for milk samples after UV light continuous (UVC) treatment. Error bars correspond to a 95% confidence interval. Different letters (a–d) between time points within each graph indicate means that are statistically different ($P < 0.05$). Asterisks (*) indicate P -values for the mixed linear model used to analyze the mean bacterial count (log cfu/mL) difference between all sampling time points. PT = UV lamp off and after the pump had been on for 2 min and 30 s; T1 = UV lamp on and after the pump had been on for 2 min and 30 s; T2 = UV lamp on and after the pump had been on for 5 min; T3 = UV lamp on and after the pump had been on for 7 min and 30 s.

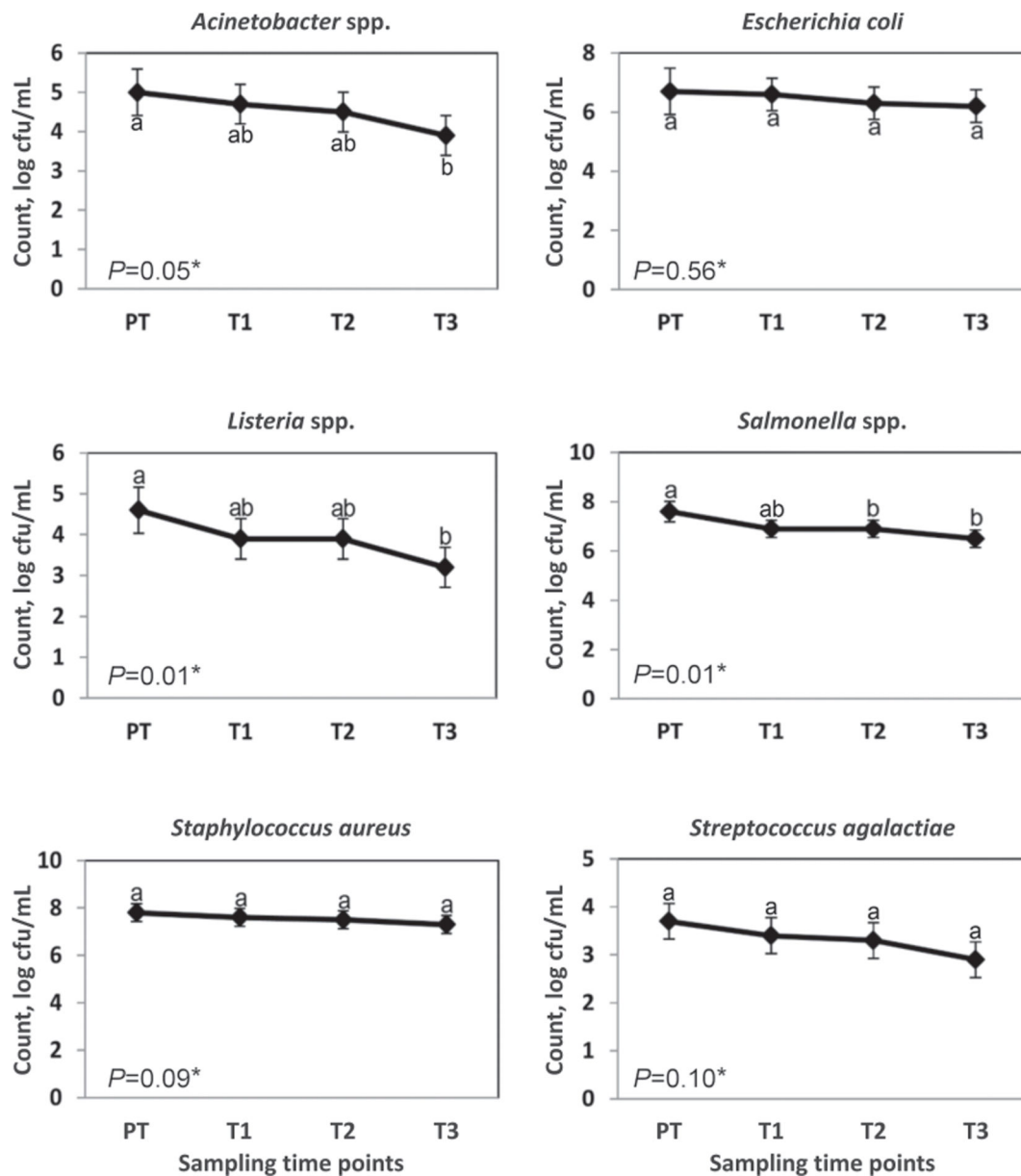


Figure 4.

Counts (mean log cfu/mL) for colostrum samples after UV light continuous (UVC) treatment. Error bars correspond to a 95% confidence interval. Different letters (a and b) between time points within each graph indicate means that are statistically different ($P < 0.05$). Asterisks (*) indicate P -values for the mixed linear model used to analyze the mean bacterial count (log cfu/mL) difference between all sampling time points. PT = UV lamp off and after the pump had been on for 2 min and 30 s; T1 = UV lamp on and after the pump had been on for 2 min and 30 s; T2 = UV lamp on and after the pump had been on for 5 min; T3 = UV lamp on and after the pump had been on for 7 min and 30 s.

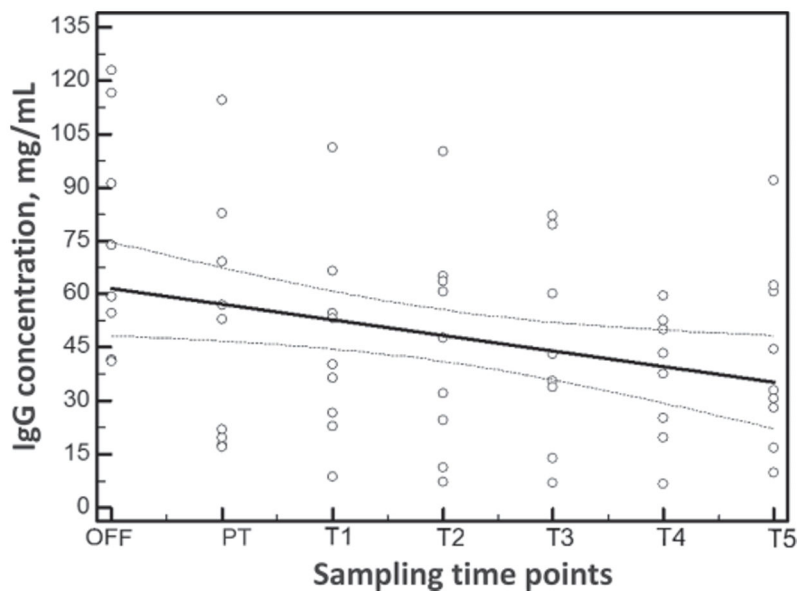


Figure 5.

Simple linear scatter plot indicating the negative association of treatment of colostrum with UV light continuous (UVC) and IgG concentration ($P = 0.019$). A 95% confidence interval is delimited by the trace lines. To evaluate the effect of UVC on colostrum IgG, samples were collected at 7 time points: (1) UV lamp and pump both off and after mixing the milk or colostrum manually with a sterile rod for 1 min (OFF), (2) UV lamp off and after the pump had been on for 2 min and 30 s (PT), (3) UV lamp on and after the pump had been on for 2 min and 30 s (T1), (4) UV lamp on and after the pump had been on for 5 min (T2), (5) UV lamp on and after the pump had been on for 7 min and 30 s (T3), (6) UV lamp on and after the pump had been on for 10 min (T4), and (7) UV lamp on and after the pump had been on for 12 min and 30 s (T5).

Table 1

Bacterial species, strain identification (ID), and description of bacteria used to inoculate whole milk and colostrum

Bacteria	Strain ID	Description ¹
Gram-negative bacteria		
<i>Salmonella</i> Typhimurium	ATCC 14028	ATCC strain
<i>Salmonella</i> Typhimurium	N001	Resistance to AMPI/KAN/STR/STX/TET
<i>Salmonella</i> Typhimurium	MDR001	Resistance CRO/CHL/NAL/TET/STR
<i>Escherichia coli</i>	ATCC 25922	ATCC strain
<i>E. coli</i>	34405-1	Resistance to STX/TET/AMPI/STR and EAEC
<i>E. coli</i>	34583-1	EAEC/EHEC and resistance to CEFT/AMC/STX/TET/AMPI/STR
<i>Acinetobacter baumannii</i>	ATCC 19606	ATCC strain
<i>A. baumannii</i>	4628	Resistance to AK/AMS
<i>A. baumannii</i>	5076	Resistance to AK
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	ATCC 27708	ATCC strain
<i>Staph. aureus</i>	5445	Methicillin-resistant <i>Staph. aureus</i> (MRSA)
<i>Staph. aureus</i>	5480	MRSA
<i>Streptococcus agalactiae</i>	SAG2	Resistant to STX
<i>Strep. agalactiae</i>	SAG18	Resistant to STX
<i>Listeria innocua</i>	ATCC 33090	ATCC strain and surrogate for <i>Listeria monocytogenes</i>
<i>Mycobacterium smegmatis</i>	mc2155	Surrogate for <i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i>

¹AK = amikacin; AMC, amoxicillin-clavulanic acid; AMPI = ampicillin; AMS = ampicillin-sulbactam; ATCC = American Type Culture Collection; CEFT = ceftiofur; CHL = chloramphenicol; CRO = ceftriaxone; EAEC = enteroaggregative *E. coli*; EHEC = enterohemorrhagic *E. coli*; KAN = kanamycin; NAL = nalidixic acid; STR = streptomycin; STX = sulfamethoxazole-trimethoprim; TET = tetracycline.

Table 2

Bacterial species culture media and growth conditions

Bacterial species	Broth culture media and growth conditions ¹	Recovery and enumeration media and growth conditions ²
<i>Salmonella</i> Typhimurium	LB broth for 24 h at 37°C (Barrow et al., 1996)	CHROMagar ³ <i>Salmonella</i> for 24 h at 37°C
<i>Escherichia coli</i>	LB broth for 24 h at 37°C (Zhang et al., 2011)	CHROMagar <i>E. coli</i> for 24 h at 37°C
<i>Acinetobacter baumannii</i>	BHI broth for 24 h at 37°C (Viazis et al., 2008)	CHROMagar <i>Acinetobacter</i> for 24 h at 37°C
<i>Staphylococcus aureus</i>	LB broth for 24 h at 37°C (Zhang et al., 2011)	CHROMagar <i>Staphylococcus</i> for 24 h at 37°C
<i>Streptococcus agalactiae</i>	BHI broth for 24 h at 37°C (Viazis et al., 2008)	CHROMagar StrepB for 24 h at 37°C
<i>Listeria innocua</i>	BHI broth for 24 h at 37°C (Viazis et al., 2008)	CHROMagar <i>Listeria</i> for 48 h at 37°C
<i>Mycobacterium smegmatis</i>	LB with 0.05% Tween 80 for 72 h at 37°C (Anuchin et al., 2009; Haydel et al., 2012)	MB 7H10 with 10% ADC and 5 mL of 100% glycerol/L for 72 h at 37°C (Singh and Reyrat, 2009)

¹LB = Luria-Bertani broth; BHI = brain-heart infusion broth.

²MB = Middlebrook culture media; ADC = oleic acid-albumin-dextrose-catalase.

³CHROMagar, Paris, France.