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Effect of Selective Decontaminants on Quality of Buffalo Rumen

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A B S T R A C T

A study to assess the effect of dipping buffalo rumen samples in 2% Lactic Acid (LA) for 10 sec., 150 ppm Chlorinated water (CW) for 5 sec and 10% Trisodium Phosphate (TSP) for 5 min., on its various quality characteristics was conducted. Tap water washed rumen pieces were used as control. Both the control and treated samples were stored at refrigerated temperature (4±1°C) until visible signs of spoilage observed as indicated by sensory scores. The treated and control rumen samples were analyzed for the effect of decontaminants on various inherent microorganisms, inoculated pathogens, sensory and physio-chemical characteristics at regular intervals. Result revealed that 2% LA and 10% TSP were equally and significantly more effective ($p<0.01$) in reducing total viable, *Coliforms*, Staphylococcal and *E. coli* counts than 150 ppm CW, both immediately after treatment as well as during the storage at refrigerated (4±1°C) condition. Sensory scores for odour and colour were significantly lower ($p<0.01$) for all treated samples than control. The colour scores did not differ significantly among the treatments. However, 10% TSP and 150 ppm CW treatments resulted in significantly higher odour scores than 2% LA treatment. 10% TSP treatment was most effective in reducing *L. monocytogenes* count followed by 150 ppm CW and 2% LA and the inhibitory effect was maintained throughout the storage period. Conversely, 10% TSP treatment was most effective in reducing *Salmonella* count only on day zero of refrigerated storage; upon subsequent storage, 10% TSP and 2% LA were equally effective in reducing Salmonella count. 2% LA and 10% TSP were equally and significantly effective in reducing Aermonas count throughout the storage period studied. 10% TSP was most effective in reducing verotoxigenic *E. coli* count whereas it was 2% LA with respect to Campylobacter count.

Introduction

During slaughter and processing of food animals, all potentially edible tissues including organ meats are subjected to contamination from a variety of sources within and outside the animal (Ayres, 1955). Due to readily available nutrients and poor hygienic conditions during handling, collection and processing, offal generally spoils faster than meat from skeletal tissues. Further, pathogenic microorganisms are the major safety concern for the meat industry. In general, microbial contamination of offal results in economic loss and may lead to public health concerns when consumed or used as ingredient in processed meat products. If microbes on the surface of meat and offal could be eliminated or substantially reduced immediately after slaughter, the risk of cross contamination during processing would be substantially reduced.

Several methods have been developed for bacterial reduction on carcasses which include trimming (Prasai *et al.,* 1995a, b), water washing **(**Hardin *et al.,* 1995; Reagan *et al*., 1996**)**, hot water spraying (Smith and Graham, 1978), steam pasteuring (Phebus *et al.,* 1997), as well as sanitizing by chemicals such as organic acids **(**Dorsa *et al.,* 1997b), chroline compounds **(**Kotula *et al.,* 1974**),** polyphosphates **(**Dickson *et al.,* 1994**)** and disinfectants. Although there are several studies on decontamination of carcasses, only limited reports are available on decontamination of offal (Patterson and Gibbs, 1979; Woolthuis *et al.,* 1984**;** Delmore *et al.,* 2000**)**. These studies on carcasses and offal indicate that an efficient method of surface decontamination provides an additional barrier protection beyond low temperature control and, thereby offers substantial advantages in terms of food safety, spoilage and economics. Considering these points, in the present study, lactic acid, sodium hypochlorite and trisodium phosphate were used to evaluate their efficacy as a decontaminant by determining their effect on intrinsic microbial flora, sensory and physiochemical characteristics of buffalo rumen. Apart from this, the effects of these decontaminants were also tested on various food borne pathogens of public health importance by artificial spiking studies.

Materials and Methods

Collection and packaging of buffalo rumen samples

Buffalo rumen samples were collected, on separate days, from offal market of Bareilly (India) within 3 to 4 hours of slaughter. Collected samples were placed immediately into sterile polyethylene bags and held under refrigerated condition during their transport to the laboratory.

Decontaminants used

Immersion treatments applied to reduce contamination of the rumen samples included Lactic acid (LA) (2% vol/vol, pH 2.5, 37 °C prepared from LA, Himedia), chlorine (Sodium hyphochlorite, SHC, 0.015% vol/vol or 150 ppm CW, 37 °C, pH adjusted to 6.5, Ranbaxy) and Trisodium phosphate (TSP) (10% wt/vol, pH 12.65, 45 °C, prepared from trisodium phosphate dodecahydrate, Merck). Tap water (sterile) washed rumen samples were maintained as control. Concentration-contact time combination for each decontaminant utilised in the present study was based on the results obtained through preliminary experiments (Selvan *et al*. 2007a, b & c).

Bacterial strains and culture conditions

Listeria monocytogenes MTCC 1143 4(b), *Aeromonas hydrophila* MTCC 646, *Salmonella Typhimurium*, field isolates of

Verotoxigenic *E. coli* and *Campylobacter jejuni* were obtained from the Division of Veterinary Public Health, Indian Veterinary Research Institute (IVRI, India). Except for *C. jejuni*, stock cultures of each pathogen were grown aerobically in Brain Heart Infusion broth (BHI) at 37°C for 24 h (*Salmonella Typhimurium* and *L. monocytogenes*) or at 35 °C for 24 h (Verotoxigenic *E. coli*) or at 30°C for 24 h (*Aeromonas hydrophila*) for the preparation of inoculum whereas stock cultures of *C. jejuni* were grown in BHI broth supplemented with 6% defibrinated horse blood and incubated at 42 °C for 48 h under microaerophilic conditions. 10 ml of each overnight grown culture (pathogen) was individually centrifuged at 8000 rpm for 10 min at 4°C to harvest cells. The harvested cells were washed twice with Phosphate Buffer Saline (PBS) by centrifugation at 5000 g for 10 min. Final cell pellet was suspended in the same buffer and the concentrations of cells were adjusted to 10^4 cells/ml using Mcfarland's turbidity method. Viable cell counts from individual dilutions were confirmed by retrospective spread plating onto BHI agar plates.

Effect of decontaminants on intrinsic microbial flora, sensory and physio-chemical characteristics

Decontamination procedure

On the day of collection, rumen samples obtained from each animal was hygienically portioned into pieces of 100 g and the pieces were divided into four groups each containing four. Rumen pieces from the three groups were separately immersed in solutions of 2% LA, 150 ppm CW and 10% TSP for 10 sec, 5 sec and 5 min, respectively, whereas rumen pieces from fourth group were washed with tap water and maintained as control(s). The ratio of rumen pieces and dipping/washing solutions was 1:4 (wt/vol). Treated and control pieces were drained and packed separately in sterile polyethylene bags. Then, they were stored at refrigerated, aerobic (4±1°C) conditions until discernible signs of spoilage appeared as indicated by appearance and odour changes. The control and treated pieces were analysed 45 min after the treatments as well as on day 4, 8 and 12 of refrigerated storage for certain microbial, sensory and physio-chemical characteristics. One piece from each group was drawn for analysis on each occasion.

Microbiological analysis

Microbiological quality of control and treated rumen pieces was evaluated based on the counts of total viable bacteria (Total viable count), *Coliforms, Staphylococcus and Escherichia coli.* All microbial groups except *Escherichia coli* were determined by pour plate method, following the procedures of APHA (1984) whereas *E. coli* counts were determined using spread plate method. Five grams from each piece was aseptically blended with 45 ml of 0.1% sterile peptone water in a pre-sterilised mortar. Decimal dilutions were prepared from the blended samples using sterile 0.1% peptone water. For the counts, one ml from each of the serially diluted homogenate was inoculated in duplicate, using appropriate growth media, in sterile petri dishes by pour plate method. Inocula on plate count agar were incubated at 37±1°C for 48 h under aerobic conditions to assess the total viable counts. Enumeration of coliforms was carried out on Violet Red Bile Agar incubated at 37±1 °C for 24 h aerobically. Staphylococci were enumerated on Baird Parker agar supplemented with egg yolk tellurite emulsion incubated at 37±1°C for 48 h under aerobic condition. A quantity of 0.1 ml of appropriate decimal dilutions plated on Mac Conkey's agar using spread plate technique were incubated at 35±1°C for 48 h to enumerate *E. coli* colonies. The

presumptive colonies were determined by counting the number of pinkish sharp colonies with about 0.5 mm diameter. Colonies judged to be borderline cases were also counted. The average numbers of colonies were expressed as $\log_{10}$cfu/g of rumen samples.

Sensory analysis

The effect of treatments on the colour and odour of rumen pieces were noted during the refrigerated, aerobic (4±1 °C) storage by a sensory evaluation panel comprising post graduate students and scientists of Livestock Products Technology division (IVRI, India). The six point sensory scale as described by Anna Anandh (2001) was used for scoring colour and odour of rumen pieces with modifications.

Physio chemical analysis

Control and treated rumen pieces were evaluated for physiochemical characteristics like pH, Extract Release Volume (ERV) and Titratable acidity (only for 2% LA treated samples) during refrigerated storage at (4±1°C). The pH-value of the buffalo rumen pieces were determined by homogenising 10 g of sample with 50ml distilled water in an Ultra Turrex (IKA, Model T-25, Germany) homogeniser for one min at 3000 rpm. pH of the suspension was recorded by immersing the combined glass electrode of digital pH merer (Model CP-901, Century Instruments Ltd., India).The method described by Konecko (1979) was used with some modifications for estimation of titratable acidity. Similarly, Extract Release Volume (ERV) was estimated using the procedure described by Jay, 1964.

Effect of decontaminants on inoculated pathogens

Experimental inoculation studies were carried out to compare the effect of decontaminants on pathogens (*Listeria monocytogenes*, *Aeromonas hydrophila*, *Salmonella Typhimurium*, Verotoxigenic *E. coli* and *Campylobacter jejuni*) inoculated in buffalo rumen samples. For this purpose, randomly collected buffalo rumen samples were screened for the presence of above mentioned pathogens and the negative samples were used for respective experimental inoculation studies. To compare the effect of decontaminants on Verotoxigenic *E. coli*, rumen pieces were steamed for 10 min at 100°C, prior to use in the study.

Inoculation of samples

Buffalo rumen samples negative for *L. monocytogenes* was portioned into pieces of five gram and were divided into four groups each containing five. Then, the pieces were separately placed in sterile polypropylene sachets/HiDispo bags (Hi media). Each piece of rumen in all groups was individually inoculated with *L. monocytogenes* at a concentration of 10^4 cells per gram of sample. After inoculation, the pieces were kept for 30 min at room temperature to allow the bacteria to attach. All the procedures were carried out aseptically to avoid any contamination.

Decontamination procedure

Listeria inoculated rumen pieces from the three groups were separately immersed in solutions of 2% LA, 150 ppm CW and 10% TSP for 10 s, 5 s and 5 min, respectively whereas rumen pieces from fourth group were washed with tap water and maintained as control(s).

After immersion, pieces (both control and treated) were drained and packed independently in sterile polyethylene bags. Then, they were stored under refrigerated, aerobic (4±1°C) conditions. The control and treated samples were evaluated 45 min after

the treatment as well as on day 3, 6, 9 and 12 of refrigerated storage for *L. monocytogenes* count. One sample from each group was drawn for analysis on each occasion.

Microbiological analysis

Each inoculated rumen pieces was aseptically blended with 45 ml of 0.1% sterile peptone water in a pre-sterilised mortar. Decimal dilutions in sterile 0.1% peptone water were prepared from the blended samples and 0.1 ml volumes were spread in duplicate onto Dominiguez-Rodriguez agar and incubated at 37 °C for 48 h.

The typical colonies of *L. monocytogenes* (appeared as greenish yellow, glistening, iridescent and pointed colonies of about 0.5 mm surrounded by diffused black zone of aesculin by hydrolysis) were counted and expressed as $\log_{10}$cfu/g of sample. Similar procedure was followed in duplicate using other pathogens with the inoculation level of 10^4 cells/g of sample.

Then, they were enumerated in appropriate growth media using spread plate technique. Enumeration of *Salmonella Typhimurium*, Verotoxigenic *E. coli* and *Aeromonas hydrophila* were carried out on Bismuth Sulphite agar at 37 °C for 24 h, Mac Conkey agar at 35 °C for 24 h and Ampicillin Dextrin agar at 37 °C for 24 h, respectively. Colonies of *C. jejuni* were enumerated in modified Park and Sanders agar at 43°C for 48 h under microaerophilic conditions. Minimum five trials were conducted using each pathogen.

Statistical analysis

The data obtained were analysed using standard statistical procedures (Snedecor and Cochran, 1994). Analysis of variance (ANOVA) procedure was used to determine the significant difference ($p \leq 0.01$) among means obtained for different treatments.

Results and Discussion

Effect on intrinsic microbial flora

Total viable count (TVC), *Coliforms*, Staphylococcal and *E. coli* counts of treatments were significantly different from tap water washed control (Tab. 1). Among treatments, 2% LA and 10% TSP were equally and significantly more effective ($p<0.01$) in reducing total viable, *Coliforms*, Staphylococcal and *E. coli* counts than 150 ppm CW, both immediately after treatment as well as during the storage at refrigerated (4±1°C) condition.

Our results are in accordance with Delmore *et al.*, (2000) who also observed similar reduction in aerobic plate, Coliforms and *E. coli* counts of beef cheek meats immersed in 2% LA for 10 s as well as that immersed in 12% TSP for 10 s.

Effect on sensory characteristics

The effect of treatments on the colour and odour of buffalo rumen samples were noted 45 min after the treatments and on day 4 of refrigerated, aerobic (4±1 °C) storage. As the control(s) have spoiled, sensory evaluation could not be conducted on day 8 and 12 of storage. Sensory scores for odour and colour were significantly lower ($p<0.01$) for all treated samples than control. The colour scores did not differ significantly among the treatments. However, 10% TSP and 150 ppm CW treatments resulted in significantly higher odour scores than 2% LA treatment (Tab. 2). Ravindranath (1994) also observed reduction in odour scores of buffalo meat treated with 2% LA. Saoji *et al.*, (1990) also observed reduction in odour scores of buffalo meat streaks prepared from 4% LA treated buffalo carcasses. Snijders *et al.*, (1979) also observed slight reversible discolouration in beef carcasses sprayed with 1% LA.

Table.1 Effect of different decontaminants on microbial quality of buffalo rumen during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	Total viable count				
0th day	6.17±0.08^{dA}	5.04±0.04^{dC}	5.33±0.08^{dB}	5.14±0.04^{dC}	**5.42±0.11^{d}**
4th day	6.83±0.06^{cA}	5.63±0.06^{cC}	5.93±0.06^{cB}	5.76±0.05^{cBC}	**6.04±0.11^{c}**
8th day	7.60±0.09^{bA}	6.59±0.05^{bC}	7.05±0.03^{bB}	6.63±0.03^{bC}	**6.97±0.10^{b}**
12th day	8.32±0.10^{aA}	7.37±0.07^{aC}	7.74±0.05^{aB}	7.43±0.05^{aC}	**7.71±0.09^{a}**
Treatment Mean±SE	**7.23±0.19^{A}**	**6.16±0.21^{C}**	**6.51±0.22^{B}**	**6.24±0.20^{C}**	
	Coliform count				
0th day	5.30±0.08^{dA}	3.98±0.14^{cC}	4.67±0.06^{dB}	4.04±0.13^{dC}	**4.50±0.13^{d}**
4th day	5.74±0.07^{cA}	4.38±0.15^{bcC}	5.22±0.05^{cB}	4.46±0.13^{cC}	**4.95±0.14^{c}**
8th day	6.15±0.08^{bA}	4.78±0.14^{bC}	5.67±0.05^{bB}	4.93±0.13^{bC}	**5.38±0.14^{b}**
12th day	6.59±0.07^{aA}	5.23±0.15^{aC}	6.06±0.03^{aB}	5.32±0.14^{aC}	**5.80±0.14^{a}**
Treatment Mean±SE	**5.95±0.11^{A}**	**4.59±0.13^{C}**	**5.40±0.12^{B}**	**4.69±0.13^{C}**	
	Staphylococcal count				
0th day	5.79±0.05^{dA}	4.87±0.10^{dC}	5.27±0.09^{dB}	4.92±0.08^{dC}	**5.21±0.09^{d}**
4th day	6.16±0.04^{cA}	5.20±0.10^{cC}	5.71±0.08^{cB}	5.33±0.07^{cC}	**5.60±0.09^{c}**
8th day	6.57±0.05^{bA}	5.62±0.10^{bC}	6.13±0.08^{bB}	5.74±0.05^{bC}	**6.02±0.09^{b}**
12th day	6.98±0.05^{aA}	6.04±0.11^{aC}	6.58±0.09^{aB}	6.16±0.05^{aC}	**6.44±0.09^{a}**
Treatment Mean±SE	**6.38±0.10^{A}**	**5.43±0.11^{C}**	**5.92±0.12^{B}**	**5.53±0.11^{C}**	
	***E. coli* count**				
0th day	3.11±0.10^{cA}	2.31±0.04^{cC}	2.64±0.02^{bB}	2.29±0.05^{dC}	**2.59±0.08^{d}**
4th day	3.30±0.09^{bcA}	2.45±0.03^{bcC}	2.81±0.12^{abB}	2.44±0.05^{cC}	**2.75±0.09^{c}**
8th day	3.52±0.09^{abA}	2.61±0.10^{abC}	2.96±0.12^{abB}	2.59±0.03^{bC}	**2.92±0.10^{b}**
12th day	3.67±0.09^{aA}	2.75±0.10^{aC}	3.13±0.12^{aB}	2.75±0.03^{aC}	**3.07±0.10^{a}**
Treatment Mean±SE	**3.40±0.07^{A}**	**2.53±0.05^{C}**	**2.88±0.06^{B}**	**2.52±0.04^{C}**	

Means with common superscripts in a row (capital alphabets) and in a column (small alphabets) for a given organism do not differ significantly ($p<0.01$).

Delmore *et al.* (2000) also reported that immersion of variety meats in decontaminants had greater effect on the colour than spraying. Similarly, Capita *et al.* (2000) observed reduction in odour, colour and overall acceptability scores of chicken thighs treated with 12% TSP for 15 min.

Effect on pH, Weight loss/gain, ERV and titratable acidity

The effect of treatments on pH, weight loss/gain, ERV and titratable acidity (for LA treated samples only) are presented in Table 3. pH values of treatments differed significantly ($p<0.01$) from that of control.

Among treatments, 2% LA and 10% TSP treatments resulted in significantly lower and higher ($p<0.01$) pH-values, respectively.

Dorsa *et al.,* (1998) also observed similar patterns of lower and higher surface pH values of beef carcass tissues (BCT) washed with 2% LA and 12% TSP, respectively. Similarly, King*et al.,* (2012) also observed the lower pH-values in all the pig variety meats immediately after spraying with LA. Extract release volume of treatments were significantly ($p<0.01$) different from that of control. Among treatments, 2% LA treatment elicited significantly ($p<0.01$) higher ERV. Significant reduction of pH by LA treatment might have contributed to increase in the ERV by reducing hydration of proteins.

Conversely, TSP would have favoured the hydration of proteins by increasing the pH of treated samples thereby led to decrease in ERV. Titratable acidity values (ml of 0.01N NaOH/g of sample) for control and 2% LA treated samples were 0.62 and 0.81, respectively and was higher in LA treated rumen samples. Factors that were responsible for lowering the pH would have contributed to the increase in titratable acidity.

Table.2 Effect of different decontaminants on sensory characteristics of buffalo rumen during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	Odour score				
0th day	5.00±0.00^{A}	4.02±0.12^{C}	4.40±0.10^{B}	4.31±0.09^{B}	**4.43±0.06**
4th day	5.00±0.00^{A}	4.36±0.10^{C}	4.64±0.09^{B}	4.52±0.10^{BC}	**4.63±0.05**
Treatment Mean±SE	**5.00±0.00^{A}**	**4.19±0.08^{C}**	**4.52±0.07^{B}**	**4.42±0.07^{B}**	
	Colour score				
0th day	5.00±0.00^{A}	4.05±0.22^{B}	4.33±0.11^{B}	4.26±0.15^{B}	**4.41±0.08**
4th day	5.00±0.00^{A}	4.31±0.14^{B}	4.43±0.10^{B}	4.38±0.13^{B}	**4.53±0.06**
Treatment Mean±SE	**5.00±0.00^{A}**	**4.18±0.13^{B}**	**4.38±0.07^{B}**	**4.32±0.10^{B}**	

Means with common superscripts in a row (capital alphabets) do not differ significantly ($p<0.01$).

Table.3 Effect of different decontaminants on physicochemical parameters of buffalo rumen during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	pH				
0th day	6.50±0.06^{dB}	5.37±0.08^{dD}	6.27±0.03^{dC}	9.32±0.02^{dA}	**6.86±0.34^{d}**
4th day	6.82±0.04^{cA}	6.37±0.06^{cC}	6.60±0.04^{cB}	6.39±0.05^{cC}	**6.55±0.05^{c}**
8th day	7.19±0.04^{bA}	6.68±0.03^{bC}	7.08±0.04^{bB}	6.77±0.03^{bC}	**6.93±0.05^{b}**
12th day	7.55±0.06^{aA}	7.30±0.03^{aC}	7.34±0.02^{aB}	7.14±0.03^{aC}	**7.27±0.05^{a}**
Treatment Mean±SE	**7.02±0.09^{B}**	**6.36±0.14^{D}**	**6.82±0.10^{C}**	**7.41±0.26^{A}**	
	Weight loss/gain				
0th day	101.44±0.54^{aB}	100.53±0.16^{aB}	100.98±0.39^{aB}	104.60±0.40^{aA}	**101.89±0.41^{a}**
4th day	98.90±0.38^{bB}	98.87±0.19^{bB}	99.48±0.55^{bAB}	100.44±0.32^{bA}	**99.42±0.23^{b}**
8th day	97.31±0.30^{A}	96.77±0.24^{c}	97.47±0.42^{c}	98.45±0.58^{c}	**97.50±0.23^{c}**
12th day	95.73±0.27^{d}	95.80±0.19^{d}	96.12±0.40^{c}	96.23±0.44^{d}	**95.97±0.16^{d}**
Treatment Mean±SE	**98.34±0.52^{B}**	**97.99±0.43^{B}**	**98.51±0.47^{B}**	**99.93±0.74^{A}**	
	Extract Release Volume (ERV)				
0th day	14.20±0.28^{aC}	24.36±0.52^{aA}	16.76±0.52^{aB}	10.28±0.07^{bD}	**16.40±1.19^{a}**
4th day	08.96±0.46^{bB}	15.74±0.29^{bA}	10.10±0.87^{bB}	14.80±0.35^{aA}	**12.40±0.71^{b}**
8th day	01.84±0.37^{cB}	10.82±0.66^{cA}	02.20±0.36^{cB}	09.52±0.44^{bA}	**6.10±0.97^{c}**
12th day	0.18±0.08^{dC}	04.04±0.47^{dA}	0.44±0.14^{dC}	01.98±0.40^{cB}	**1.66±0.38^{d}**
Treatment Mean±SE	**6.30±1.30^{D}**	**13.74±1.71^{A}**	**7.38±1.52^{C}**	**9.15±1.07^{B}**	
	Titratable acidity				
0th day	0.66±0.03	0.85±0.03^{a}	-	-	**0.75±0.04^{a}**
4th day	0.62±0.03	0.82±0.03^{ab}	-	-	**0.72±0.04^{ab}**
8th day	0.60±0.03	0.80±0.02^{ab}	-	-	**0.70±0.04^{ab}**
12th day	0.58±0.02	0.77±0.02^{b}	-	-	**0.67±0.04^{b}**
Treatment Mean±SE	**0.62±0.01**	**0.81±0.01**	-	-	

Means with common superscripts in a row (capital alphabets) and in a column (small alphabets) do not differ significantly.

Table.4 Effect of different decontaminants on pathogenic organisms inoculated in buffalo rumen during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	***Listeria monocytogenes* count**				
0th day	4.22±0.03^{eA}	3.82±0.05^{eB}	3.44±0.09^{eC}	2.34±0.05^{eD}	**3.46±0.16^{e}**
3rd day	4.72±0.03^{dA}	4.36±0.10^{dB}	3.94±0.06^{dC}	3.01±0.06^{dD}	**4.01±0.15^{d}**
6th day	5.32±0.04^{cA}	4.82±0.09^{cB}	4.50±0.06^{cC}	3.65±0.05^{cD}	**4.57±0.14^{c}**
9th day	5.96±0.03^{bA}	5.25±0.08^{bB}	5.00±0.05^{bC}	4.27±0.06^{bD}	**5.12±0.14^{b}**
12th day	6.74±0.02^{aA}	5.81±0.07^{aB}	5.53±0.04^{aC}	4.94±0.06^{aD}	**5.76±0.15^{a}**
Treatment Mean±SE	**5.39±0.18^{A}**	**4.81±0.14^{B}**	**4.48±0.15^{C}**	**3.64±0.19^{D}**	
	***Salmonella* count**				
0th day	3.49±0.04^{aA}	2.82±0.06^{aB}	3.44±0.09^{aA}	2.54±0.04^{aC}	**3.07±0.10^{a}**
3rd day	3.18±0.05^{bA}	2.55±0.06^{bB}	3.18±0.03^{bA}	2.50±0.03^{abB}	**2.85±0.08^{b}**
6th day	2.93±0.04^{cA}	2.50±0.05^{bcB}	2.94±0.03^{cA}	2.46±0.04^{abB}	**2.71±0.05^{c}**
9th day	2.84±0.05^{cdA}	2.42±0.04^{bcB}	2.84±0.03^{cdA}	2.43±0.04^{bB}	**2.63±0.05^{d}**
12th day	2.76±0.04^{dA}	2.34±0.04^{cB}	2.77±0.03^{dA}	2.39±0.04^{bB}	**2.57±0.05^{e}**
Treatment Mean±SE	**3.04±0.06^{A}**	**2.53±0.04^{B}**	**3.04±0.05^{A}**	**2.47±0.04^{C}**	
	***Aeromonas* count**				
0th day	3.87±0.03^{eA}	2.33±0.08^{eB}	3.71±0.05^{eA}	2.48±0.04^{eB}	**3.10±0.16^{e}**
3rd day	4.22±0.05^{dA}	2.72±0.08^{dC}	3.99±0.06^{dB}	2.85±0.05^{dC}	**3.44±0.16^{d}**
6th day	4.56±0.06^{cA}	3.10±0.09^{cC}	4.35±0.04^{cB}	3.19±0.05^{cC}	**3.80±0.15^{c}**
9th day	4.91±0.06^{bA}	3.47±0.07^{bC}	4.66±0.03^{bB}	3.52±0.04^{bC}	**4.14±0.15^{b}**
12th day	5.21±0.05^{aA}	3.80±0.07^{aC}	4.97±0.04^{aB}	3.84±0.03^{aC}	**4.46±0.15^{a}**
Treatment Mean±SE	**4.55±0.10^{A}**	**3.08±0.11^{D}**	**4.04±0.09^{B}**	**3.18±0.10^{C}**	
	Verotoxigenic *E.coli* count				
0th day	3.27±0.03^{A}	2.64±0.03^{C}	2.93±0.05^{B}	2.33±0.03^{D}	**2.79±0.08**
3rd day	2.46±0.07	<2	<2	<2	-
6th day	<2	<2	<2	<2	**<2**
9th day	<2	<2	<2	<2	**<2**
12th day	<2	<2	<2	<2	**<2**
Treatment Mean±SE	-	-	-	-	-
	***Campylobacter* count**				
0th day	2.91±0.04^{A}	2.53±0.03^{C}	2.72±0.02^{B}	2.81±0.05^{AB}	**2.74±0.04**
3rd day	<2	<2	<2	<2	**<2**
6th day	<2	<2	<2	<2	**<2**
9th day	<2	<2	<2	<2	**<2**
12th day	<2	<2	<2	<2	**<2**
Treatment Mean±SE	-	-	-	-	-

Effect on inoculated pathogens

Table 4 shows counts of *L. monocytogenes*, *Salmonella Typhimurium*, *Aeromonas hydrophila*, verotoxigenic *E. coli* and *C. jejuni* recovered from inoculated buffalo rumen samples that had undergone tap water (control) or other treatments prior to refrigerated aerobic storage at 4±1 °C. 10% TSP treatment was most effective in reducing *L. monocytogenes* count followed by 150 ppm CW and 2% LA and the inhibitory effect was maintained throughout the storage period. Conversely, 10% TSP treatment was most effective in reducing *Salmonella* count only on day zero of refrigerated storage; upon subsequent storage, 10% TSP and 2% LA were equally effective in reducing *Salmonella* count. 2% LA and 10% TSP were equally and significantly effective in reducing *Aermonas* count throughout the storage period studied. 10% TSP was most effective in reducing Verotoxigenic *E. coli* count whereas it was 2% LA with respect to *Campylobacter* count. Upon storage, both these inoculated pathogens were not detected in any of the treated samples by normal plating procedures. However, enrichment and selective plating of stored samples confirmed the presence of these pathogens. Hence, it can be concluded that treatments could not eliminate the organisms and the reduction observed during storage would be due to the effect of low temperature storage and or treatment employed. Uyttendaele *et al.*, (2004) also observed that decontamination with LA solutions (1% and 2%; wt/vol) showed the most potential to reduce *Aeromonas* spp. and to guarantee prolonged shelf-lives of fresh-cut vegetables without affecting sensory properties.

Dorsa *et al.* (1998) also observed that the populations of S. Typhimurium and *E. coli* O157:H7 inoculated on beef surface tissues were significantly reduced or brought to below detectable levels by hot water and other antimicrobial treatments including 2% LA and 12% TSP. LA treatment immediately reduced levels of all microorganisms similar to previous reports (Delmore *et al.*, 2000; King *et al.*, 2012).

Similarly, Wagenaar *et al.*, (2006) stated that Campylobacter are much more fragile and the highest concentration of Campylobacter is found on meat directly after processing. In all subsequent steps of the food chain, the concentration may stabilise but is more likely to decrease, due to death of bacteria which is analogous to the results of present study.

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