Poultry Studies

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SMT's Pure ClO_2

Pure chlorine dioxide has many qualities superior to well-known disinfectants. Our revolutionary product is more powerful, less toxic, and much easier to use than stabilized (dirty) chlorine dioxide, bleach, and acids. Pure ClO_2 is:

- many times more powerful than bleach as a disinfectant and sanitizer
- non-corrosive as compared to bleach or other disinfectants
- not a dermal sensitizer
- able to be used on food, water and food preparation surfaces
- a very powerful oxidizer: bacteria are unable to build up tolerance
- effective against wide varieties of dangerous microorganisms, including bacteria (gram negative and gram positive), viruses, spores, molds, and fungi
- compatible with a wide range of materials
- a powerful deodorizer
- able to remove bio-slime from tanks, pipes, and fluid lines

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Our products are recognized and listed by the FDA, the EPA and OMRI (Organic Materials Review Institute). For more information please visit our website: **www.selectivemicro.com**.

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Poultry Studies

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Poultry Farm Studies

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SMT's *Selectrocide* ®

Selectrocide ® is High-Quality Chlorine Dioxide that is:

- is used at a low concentration
- is recognized as organic by the Organic Materials Review Institute (OMRI)
- is gentle on materials, users, and the environment
- offers a high efficacy at a low toxicity
- can be used in liquids with a wide pH range
- leaves no residue
- Offers a much more thorough water treatment than sodium chloride, potassium chloride, chlorine, and citric acid

Proven in the Produce Market:

- *Selectrocide* ® is used on over 150 food processing lines in the US

EPA-Registered for Poultry Drinking Water:

- The following slides are the results of tests conducted while entering the poultry market

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Objectives of Testing

This series of tests was carried out to determine the impact that *Selectrocide*® would have on poultry drinking water quality and bird health in various water systems on turkey brood farms and turkey grow-out farms.

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Testing Methods

- A 65-gallon tank and a Dosatron water-driven pump were installed on two separate poultry farms (a turkey brood farm and a turkey grow-out farm) to monitor the effects which the addition of *Selectrocide*® would have on poultry drinking water quality and bird health
- *Selectrocide*® was applied to wells, tanks, and poultry houses at location-appropriate concentrations. Water samples from each of the locations were tested at set intervals.
- Our testers measured the effect of *Selectrocide*® on poultry drinking water by recording colony-forming units (CFUs) of bacteria present in the water before and after *Selectrocide*® application
- Over the duration of the trial, our testers also monitored the mortality rate (number of deaths / total number of turkeys) of the turkey populations on the brood farms as well as the livability rate (number of turkeys survived / initial turkey population), and feed conversion rate (pounds of feed consumed / pounds of harvested turkey meat) of the turkey populations on the grow-out farms. These statistics were then compared to ratios recorded when chlorine was used as a control method for treating the poultry' s water.

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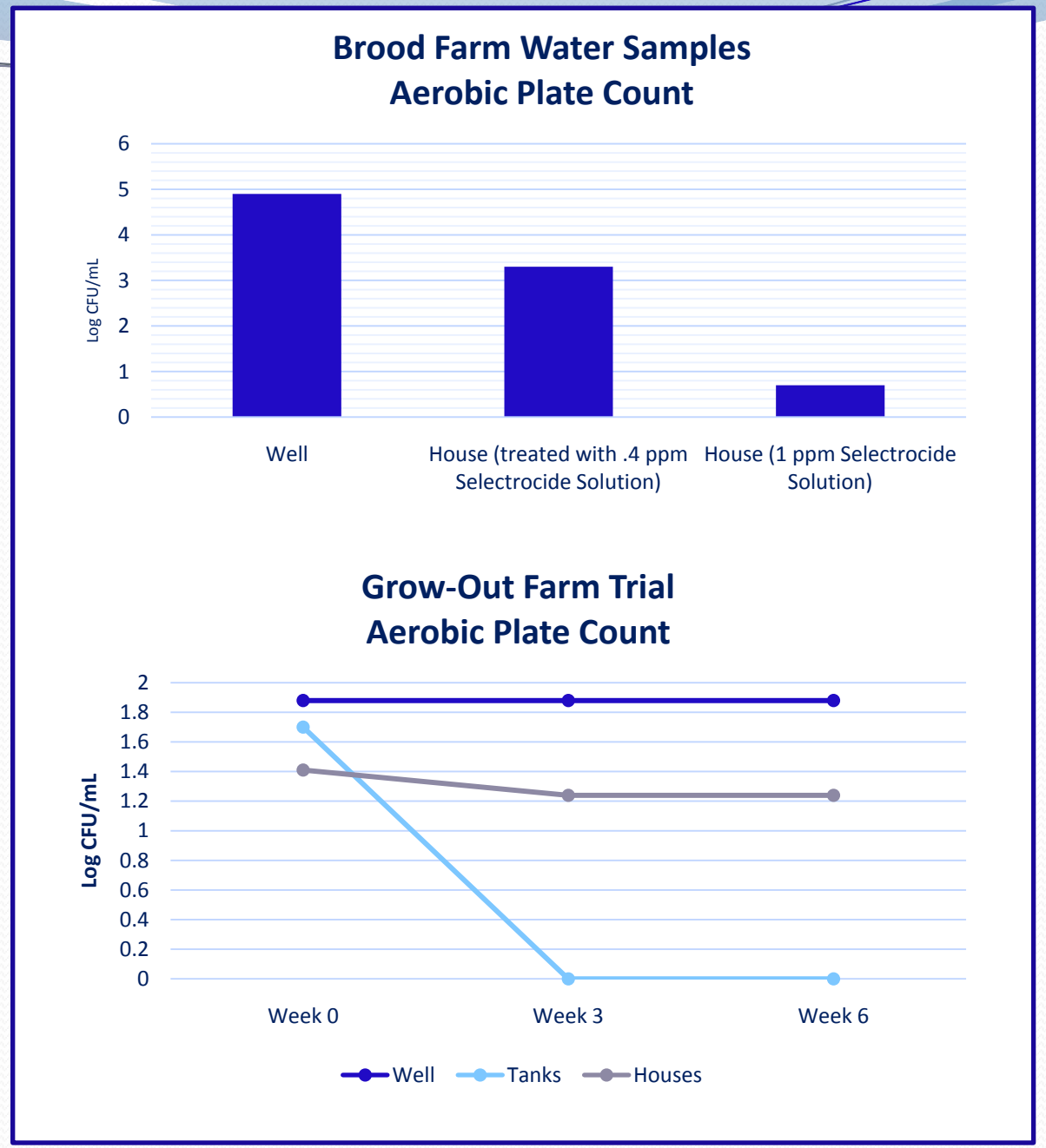
Results

- At a 1 ppm concentration, the poultry house water on brood farms recorded a >99.99% reduction in its aerobic plate counts
- During the grow-out farm trial, all storage tanks tested negative for living organisms after initial setup of the *Selectrocide*® system
- All *Selectrocide*®-treated tank and water line samples tested negative for coliform bacteria and had reduced plate counts of aerobic bacteria. All water tanks tested even had aerobic plate counts of 0.
- *Selectrocide*® at 0.4 - 0.6 ppm maintained water quality where incoming water did not have an unusually high microbial load (nearly 5 log). Higher levels of *Selectrocide*® (up to a 1 ppm concentration) were more than able to maintain water quality past this 5 log threshold.



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Effects of *Selectrocide*® Treatment on Bird Health

Brood Farm

Drinking Water

	Selectrocide®–Treated	Chlorine-Treated Water
Poultry Mortality Rate*	1.1%	1.4%

Grow-Out Farm

Drinking Water

	Selectrocide®–Treated	Chlorine-Treated Water
Livability	88.83%	79.50%
Feed Conversion	2.418	2.563

* Mortality was calculated only after *Selectrocide*® had been present in the birds' water for a full three days

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Summary

Feedback from poultry growers:

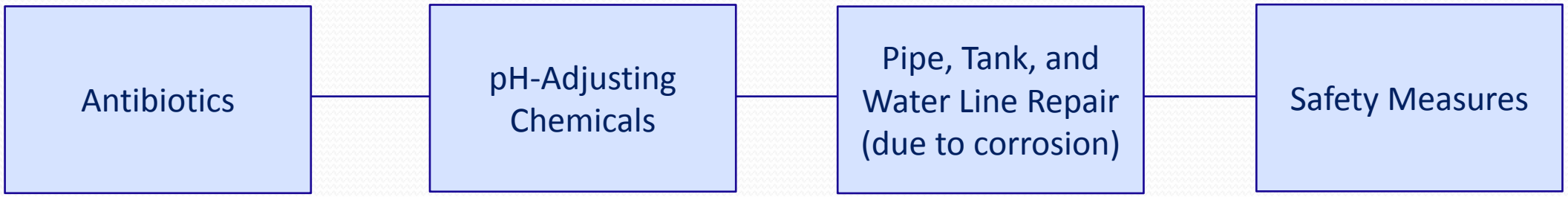
"Selectrocide® outperformed chlorine"

"Selectrocide® improved livability and overall bird health"

Selectrocide ® head-to-head with chlorine:

- *Selectrocide* ® application resulted in cleaner water and healthier birds
- Poultry growing operations using *Selectrocide*® recorded savings equal to 235% of the product's cost

Using *Selectrocide* ® also led to savings in:



Conclusion: *Selectrocide*® is an ideal water treatment option for poultry breeders, grow- out farms, and brood farms

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Poultry Meat Studies

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SMT's *Selectrocide* ®

Selectrocide ® is High-Quality Chlorine Dioxide that is:

- used at a low concentration
- recognized as organic by the Organic Materials Review Institute (OMRI)
- gentle on materials, users, and the environment
- offers a high efficacy at a low toxicity
- can be used in liquids with a wide pH range
- leaves no residue
- offers a much more thorough water treatment than sodium chloride, potassium chloride, chlorine, and citric acid

Proven in the Meat & Produce Markets:

Selectrocide ® is:

- Used on over 150 food processing lines in the US
- FDA-approved for the disinfection of fruits, vegetables, and RACs that are fruits and vegetables
- FDA-approved for the gaseous disinfection of fruits and vegetables
- EPA-registered as an antibacterial treatment of agricultural commodities (RACs), agricultural water, fruit and vegetable process water, food processing equipment, flumes, tanks, and lines

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Objectives of Testing

The purpose of this study was to evaluate the effectiveness of Selective Micro Technologies' Selectrocide® on bacterial colonies that are naturally present on or have been introduced to the surface of chicken meat and beef.

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Background

Bacterial colonies on the surfaces of meat products pose serious problems for meat suppliers and consumers. The proliferation of bacteria on the surface of meat leads to discoloration, distortion of taste and odor, and spoilage. If fact, millions of pounds of bad meat are thrown out each year due to premature spoilage.

Meat products are also one of the foods most at-risk to carry the microbes which cause foodborne illness in humans. In fact, according to the CDC, 41.6 % of cases of foodborne illness in the U.S. are attributed to pathogens which enter the body through infected processed meat.

Because the surfaces of meat products are so susceptible to the growth of bacteria, it is imperative that all meat be rid of pathogens during processing. This experiment tested the efficacy of Selectrocide®) against meat products inoculated with either *Enterococcus faecalis* (a bacteria similar to *E. coli*) or a mixture of *Salmonella* (equal parts *Salmonella typhimurium* and *Salmonella typhi*).

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Testing Methods

***Enterococcus faecalis* test**

Chicken thigh skins were removed from the exterior of the thigh and divided into four groups. The groups were then sprayed with 1 ml of stock *Enterococcus faecalis* at a concentration of 1 x 10^9 CFU/ml and allowed to attach for 2 minutes. Three of the groups were treated with a spray of either 5, 10, or 20 ppm *Selectrocide*® solution for 15 seconds, while the control group did not receive a spray. The spray was allowed to set on the skin for 1 minute to simulate commercial processing time lapse (this would be similar to immersion without a rinse) before samples were processed and incubated at 35°C for 48 hours. An identical test was carried out using beef lean round steaks in place of chicken thighs.

***Salmonella* test**

Skinless chicken breasts were split into 2 pieces and divided into four groups. The groups were then sprayed with 1 ml of the *Salmonella* mixture at a concentration 2.8 X 10^8 and allowed to attach for 2 minutes. Three of the groups were treated with a spray of either 5, 10, or 20 ppm *Selectrocide*® solution for 15 seconds, while the control group did not receive a spray. The spray was allowed to set on the skin for 2 minutes before samples were processed and incubated at 35°C for 48 hours. An identical test was carried out using beef lean round steaks in place of chicken thighs.

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Results

Study Highlights:

- The 5- and 10-ppm *Selectrocide*® spray applications most effectively *controlled Enterococcus faecalis* inoculated onto chicken breasts, reducing CFU/ml counts by 2.6 and 2.2 log respectively
- The 10-ppm spray application of *Selectrocide*® reduced *Salmonella* counts by up to 2.9 log CFU/ml
- The 5-, 10-, and 20-ppm *Selectrocide*® spray applications were effective in controlling *Salmonella* on inoculated breast meat, reducing *Salmonella* CFU/ml counts by 2.6, 2.9, and 2.6 log cycles, respectively

Conclusion:

Selectrocide® may find success if implemented into a commercial meat processing setting to reduce occurrence of bacterial contamination on carcasses as well as finished products, especially for poultry.

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Full Study Text

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Selective Micro Technologies

Power, Selectivity, Simplicity: The Ideal Biocide Solution.

Selective Micro Products

Effective Use of 5,10 and 20 ppm Solution Sprays and Dips on Beef and Poultry

The Use of Selective Micro®Clean Chlorine Dioxide on Microorganisms Inherent to Chicken and Beef surfaces, as well as Inoculated Meat and Skin Surfaces

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ABSTRACT

The purpose of this study was to evaluate effectiveness of Selective Micro®Clean products(Selectrocide®) chlorine dioxide solution on bacterial colonies inherent to chicken and beef surfaces, as well as bacteria inoculated onto the surfaces. Skins from chicken thighs (n = 64; 8 per treatment) and beef round steak portions (n = 64; 8 per treatment) were utilized for the first part of the study. Half of the samples were tested for aerobic bacteria, while the other half were inoculated with 1 ml per sample of stock *Enterococcus faecalis* and allowed to attach for 2 min to simulate normal processing conditions. Treatments were spray application of 5, 10, and 20 ppm Selective Micro®Clean solution to exterior surfaces. Serial dilutions were prepared and duplicate samples were enumerated on appropriate Petrifilm™ and incubated at 35˚C for 48 h. Colonies were counted and recorded as colony forming units/ml (CFU/ml). Dilutions exhibiting counts of 25-250 CFU/ml were utilized for data analysis. For the second portion of the study, lean chicken breast tissue portions (n = 32; 8 per treatment) were utilized. Samples were inoculated with a stock *Salmonella* cocktail of *S. typhimurium* and *S. typhi*. Samples were treated, diluted, plated, incubated and counted in the previous manner. No differences ($P > 0.05$) were found between treatments for aerobes (APC counts) found on chicken thigh skins. Treatment with Selective Micro®Clean reduced ($P < 0.001$) colonies of *E. faecalis* on inoculated thigh skins. It was found that 5 ppm was more effective than 20 ppm ($P = 0.0142$) in destroying *E. faecalis* on chicken thigh skins (6.3 log CFU/ml for 5 ppm treatment versus 7.0 log CFU/ml for 20 ppm treatment). Conversely, in beef round steak samples, *Salmonella* were reduced ($P < 0.05$) by up to 2.9 log CFU/ml with 10 ppm spray application of Selective Micro®Clean. Aerobic plate

counts seem to be inconclusive due to the higher levels of common bacteria in the controls. This is often common when using direct aerobic plate counts rather than direct microbial inoculations. Selective Micro®Clean may find success if implemented into a commercial meat processing setting to reduce occurrence of bacterial contamination on carcasses as well as finished products, especially for poultry. Studies as a direct carcass spray should be conducted primarily on beef at the 5 and 10 ppm level.

KEYWORDS: aerobes, chlorine dioxide, chicken, beef, *Salmonella, Enterococcus faecalis*

INTRODUCTION

Reducing microbial populations on meat products is always important to producers, in making safe and wholesome products and meeting quality control specifications, as well as producing a product that stays fresh in market conditions. By applying treatments to meats, microbial populations can be reduced, if not eliminated. Aerobic bacteria, or any bacteria requiring oxygen to live, are inherent to many surfaces, including meat. Aerobes in general, especially *Pseudomonas* species, cause spoilage, reduce shelf life, cause off-odors, and cause meat to turn off-colors. *Enterococcus faecalis* is a microbe that is inherent to the gastrointestinal tract of living animals and is often used in research as it acts similar to *Escherichia coli,* an indicator of sanitation and subsequent spoilage. It is almost inevitable that meat products will some how become contaminated with GI tract microbes given harvest conditions of both chicken and beef. *Salmonella* species are inherent to the gastrointestinal tract of poultry, but can also be

carried by beef and other meat animals. When consumed by people, *Salmonella* spp. and several *E. coli* spp. can cause serious food illness by bacterial intoxication.

Currently, many methods exist to decontaminate meat, including acid sprays, hot water baths, steam vacuuming, and trimming the carcass for visible contaminants. It is important to continue to search for new bactericides for meat products because bacteria can adapt to some treatments, such as acids. In work by Berry and Cutter (2000) it was demonstrated that certain strains of *Escherichia coli* have become resistant to 2% acetic acid used to decontaminate beef carcasses.

Prior to the conception of Selective Micro®Clean, the widespread use of chlorine dioxide was limited to large-scale applications such as public water systems. Chlorine dioxide was reportedly difficult to use, hard to keep in solution, and not easily transported. Generation equipment for chlorine dioxide is costly. However, Selective Micro®Clean chlorine dioxide solution can be made onsite in small quantities, stays in solution better than traditional chlorine dioxide, and is simple to dilute. Further, the nature of the product being a gas dissolved in water allows cellular penetration at a level much greater than chlorine or other chemicals and thus is not a factor in allowing bacteria to become resistant. In several prior studies, chlorine dioxide was used with success in rinse and chill water applications. However, little work could be found discussing the efficacy of chlorine dioxide applied as a topical spray to meat products. Therefore, the objective of this study was to examine the proficiency of Selective Micro®Clean as a spray in reducing bacterial populations inherent to or inoculated onto beef and chicken surfaces.

MATERIALS AND METHODS

Chicken thigh skins (n = 64, 8 per treatment) were dissected from the exterior of the thigh, and placed on portions of aluminum foil in groups of 8. Four treatment groups were tested for aerobic bacteria, while the other four were inoculated with stock *Enterococcus faecalis*. For aerobic testing, one group was tested without application of Selective Micro®Clean (control). The other three treatment groups were sprayed with Selective Micro®Clean at 5, 10, or 20 ppm for 15 seconds. The spray was allowed to set on the skin for 1 minute to simulate commercial processing time lapse (this would be similar to immersion without a rinse). Samples were then placed individually in stomacher bags, phosphate buffer solution was added to make a 1:9 dilution of skin to water, and the bags were placed in the stomacher for 30 sec at 230 rpm. Then, serial dilutions were prepared. The, 1 ml aliquots of each dilution were plated onto 3M Petrifilm for Aerobic Plate Counts. Duplicate plates were made of each dilution. Plates were then incubated at 35°C for 48 h. Colonies, exhibited as pinkish, red dots, were counted and recorded. Duplicate plates were averaged to produce and average number of colonies per dilution. Dilutions with colony counts between 25 and 250 were used for statistical analysis.

For *Enterococcus* testing, each chicken skin was inoculated with 1 ml stock (1 x 10^9 CFU/ml). The inoculum was spread evenly over the surface of the skin with a sterile glass rod and allowed to attach for 2 min. Then, as a control, one treatment group was not sprayed (control). The other 3 treatment groups were sprayed with 5, 10, or 20 ppm Selective Micro®Clean and allowed to set for 2 minutes. Serial dilutions were prepared

in the same manner previously described. Duplicate plates were made on appropriate 3M Petrifilm. Samples were incubated at 35°C for 48 h. Reddish colonies with a gas bubble around them were counted and recorded. Duplicate plate counts were averaged, and dilutions with colony counts of 15-250 were used for statistical analysis.

Beef lean round steak (n = 32) portions were used for the beef lean aerobic testing. Procedures for beef lean were identical to procedures followed for aerobic testing of chicken skin.

Lean chicken breast tissue samples (n = 32, 8 per treatment) were utilized for *Salmonella* testing. Boneless skinless chicken breasts were split into 2 pieces. One ml of stock *Salmonella* cocktail ($2.8 \times 10^8 = 8.3 \log_{10}$ *S. typhimurium* and *S. typhi*) was inoculated onto each sample, spread evenly over the surface with a sterile glass rod and allowed to attach for 2 min. One set of samples was not sprayed (control). The other 3 groups were sprayed with 5, 10, or 20 ppm Selective Micro®Clean and allowed to set for 2 min. Samples were stomached as previously described. Serial dilutions were prepared as previously described and duplicate plates per dilution were made on Bismuth sulfide agar plates. Plates were incubated at 35°C for 24 h. Colonies appearing as dark green to black spots were counted. Duplicate plates were averaged, and statistical analysis performed. Least Square Means were obtained with the General Linear Model procedure of SAS and separated by the PDIFF option at a predetermined $\alpha = 0.05$.

RESULTS AND DISCUSSION

Beef lean testing proved inconclusive (Table 1). Control samples tested had higher CFU/ml than any of the treatments. This could have been due to any number of differences in pre-purchase handling by meat markets. It has been found by other

researchers that chlorine dioxide reduced numbers of aerobes on beef.. Emswiler and others (1976) reduced APC by 1.64 log CFU/cm^2 with a 200 ppm chlorine dioxide solution sprayed onto beef carcasses. Also, Unda and others (1989) dipped ribeye steaks in a 100 ppm chlorine dioxide solution to reduce APC by 1 log CFU/cm^2.

The 5 ppm treatment did have numerically lower CFU/ml. *Enterococcus faecalis* inoculated onto chicken breasts (Table 3) were most effectively controlled by 5 and 10 ppm of Selecrocide applications which reduced CFU/ml by 2.6 and 2.2 log CFU/ml, respectively. The 20 ppm treatment, while different from controls, only had a slightly higher reduction than controls (1.5 reduction in control versus 1.9 reduction for 20 ppm ClO_2). These results are better than those reported by Thiessen and others (1984) that found a 0.80 log cycle reduction in *E. coli* on macerated chicken breast skin even with a much higher concentration of 1390 ppm ClO_2 (in chill water). Additionally, Lillard (1980) reduced fecal coliforms by 1.26 log cycles on macerated skin samples using concentrations of 400-900 ppm ClO_2 in chill water. It is possible that higher reductions were found in the Selective Micro®Clean study due to the fact that Selective Micro products are 99% pure chlorine dioxide as generated and the necessary time of less than 1 minute is adequate for intervention success. Barnes and Impey (1968) reported that the majority of bacteria on chicken are found within feather follicles and in cuts. Therefore, the bacteria may be protected from treatment on the skin, while being more susceptible to bactericide on the smooth surface of the muscle.

Any of the treatments were effective ($P < 0.05$) on *Salmonella* cocktail inoculated breast meat (2.6, 2.9, and 2.6 log cycles, respectively). In a study by Thiessen and others (1984), treatment of 1,390 ppm ClO_2 in chill water eliminated *Salmonella* incidence on

broiler carcasses from a mean occurrence of 97.3% positive carcasses in the plant. Lillard (1980) reported occurrences of *Salmonella* from 8 in 56 carcasses with no ClO_2 to 1 in 96 carcasses testing positive with concentrations of 400-600 ppm ClO_2 in chill water.

CONCLUSION

In this study, Selective Micro®Clean chlorine dioxide solution, and in particular 5 ppm was as effective or even more effective than 10 or 20 ppm at surface decontamination of pathogens and was effective in reducing bacterial colonies on meat surfaces, especially on lean chicken muscle and toward a mixture of *S. typhimurium* and *S. typhi*. Previous literature suggests that chlorine dioxide is less inhibited by organic matter than other sanitizers such as chlorine. Peeters and others (1989) indicated 0.4 mg of chlorine dioxide per liter of demineralized water significantly reduced infectivity of *Cryptosporidium parvum* oocysts from cattle feces within 15 min of contact. It has been shown to be a very effective sanitizer for food-contact surfaces to destroy organisms including *E. coli*, *Staphyloccus aureus*, as well as several fungi and viruses. Pohlman and others (2002) found a 200 ppm chlorine dioxide followed by 10% trisodium phosphate significantly reduced *E. coli*, coliforms, and aerobic plate counts on inoculated beef trimmings. This treatment also provided a redder overall color to the treated trimmings and no off-odor when compared other antimicrobial treatments including ozonated water and cetylpyridium chloride treatment. Jimenez-Villarreal and others also indicated improved or maintained instrumental and visual color, taste, cooking characteristics and lack of odor production when chlorine dioxide was used on beef trimmings before grinding. Although not indicated in this study, the authors believe different results may

have been seen with an inoculated beef lean product and if a product could have been obtained from a processing facility instead of from a retail outlet from which the handling practices of the meat sample were unknown prior to purchase. Andrews and others (2002) reported lower aerobic and psychrotrophic counts (from 1 to 4 log cycle reductions; reductions increasing with increasing initial concentrations) on shrimp and crawfish sprayed with chlorine dioxide in a high- or low-pressure prewash.

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