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An *in vitro* Evaluation of AOP Versus Chemical Based Poultry Water Sanitizers for Residual and Efficacy over Time

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Abstract: A bench top experiment was conducted to test three commercially available water sanitizing products for residuals and efficacy over time. Product A uses modified ambient oxygen to create hydroxyl ions in water and works based on an Advanced Oxidation Process (AOP). Product B is 50% stabilized hydrogen peroxide and Product C is 5.25% sodium hypochlorite. Two trials were conducted. Test solutions for Product A were made by infusing gas at 0.33 Liters Per Minute (LPM) diffusing into 1 liter of suboptimal water (microbial load >4.47 $\log_{10}$ cfu/ml; Chlorine (Cl) residual = 0 ppm). Stock solutions were first prepared for Product B and C. Stock solution for Product B was prepared at two different doses, 2 and 4 ml of the product, mixed with 128 ml of deionized water (Cl = 0 ppm) separately whereas for Product C, the stock was created mixing 4 ml of the product with 128 ml of deionized water. Test solutions for Product B and C that actually mimic the bird drinking rate were then made from each stock solution mixing at the rate of 1 ml of stock solution added to 128 ml of suboptimal water for a volume of 1L of suboptimal water. Water samples were taken for test solutions over a period of 24 h post treatment and plated for aerobic bacteria (APC) and mold counts. Residual levels for all the products were taken into account at each water sampling occasion. Results indicated that the AOP method can be used as an alternative water sanitation measure over the chemical methods.

Key words: AOP, water sanitizers, residuals, microbes

INTRODUCTION

Providing clean and safe drinking water to poultry is a basic requirement for optimizing production. One prime factor that determines the quality of drinking water is its microbial make up. Table 1 depicts acceptable levels of bacteria in colony forming units (cfu) per milliliter (ml) in drinking water for poultry operations (Watkins, 2007; Watkins, 2008).

Microbial contamination above the acceptable levels in drinking water can affect health and performance (King, 1996). Both top and bottom producing farms suffer equally from microbial contamination like *E. coli* and *Pseudomonas* (Barton, 1996). This shows water is vulnerable to microbial contamination regardless of good management practices. Health and production related issues in birds including breeders have been reported in various farms due to poor microbial water quality (Grizzle *et al*., 1997; Pearson *et al*., 1993; Gregory *et al*., 1997; Sparks, 2009). Fecal contaminated well water is a source of coliforms like *E. coli* that cause colibacillosis in poultry flocks (Jafari *et al*., 2006). Water and water systems including water tanks and drinker lines act as potential sources for *Salmonella* and *Campylobacter* (including viable but non-culturable forms) in chickens (Sparks, 2009; Waage *et al*., 1999; Johnson *et al*., 2003; Marin *et al*., 2009) and water treatment is a viable control strategy at the farm level (Doyle and Erickson, 2006; Vandeplas *et al*., 2010).

In commercial production barns, newly hatched chicks and poults are provided water supplies that are warmed to prevent chilling in birds. It has been documented that chicks less than a week old drink 5-10 gallons per thousand birds in a 24 h period (Williams *et al*., 2013). This minimal volume of water usage means water often remains in waterlines for several hours. This results in loss of efficacious sanitizer residuals which could leave birds vulnerable to microbial challenges from biofilms. It is of high interest to the poultry industry to identify alternative water sanitizers which can remain effective for extended periods of time. Various brands of water sanitizers or water line cleaners are available in the market under a few classes of disinfectants advocating their efficacy under worst case conditions. These products should be monitored for their true efficacy, applicability and cost effectiveness along with safety aspects.

Recently, the industry has observed a new water sanitation technology which disinfects water via an Advanced Oxidation Process (AOP) that modifies ambient oxygen continuously in the unit and generates

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hydroxyl ions in water which acts as the main biocidal agent. The AOP technology that was evaluated in this test produces hydrogen peroxide, H_2O_2, in water as a by-product of the formation of hydroxyl radical which serves as the residual biocide.

This study was conducted with the objective of determining baseline information on efficacies over time for different sanitizers-AOP technology, conventional chlorine and hydrogen peroxide based methods of water disinfection, using suboptimal water that has high levels of bacterial counts (>4 $\log_{10}$ colony forming units (cfu)/ml).

MATERIALS AND METHODS

Two trials were conducted *in vitro* to evaluate the commonly available water sanitizers in the market. Trial 2 is the repeated measure of trial 1, unless the differences in procedures executed in trial 2 were stated in the methods.

Products: The following products commonly available for this industry were obtained for evaluation:

1: Product A-AOP based technology with a gauge to infuse ambient oxygen at varying rates (0-40 liter per minute (LPM)
2: Product B-50% stabilized H_2O_2
3: Product C-5.25% Sodium hypochlorite

Dosing rate of the products and generation of treatments: The goal was to mimic the utilization of products as they are typically used in poultry drinking water supplies. Test water for trial 1 and trial 2 were obtained from separate poultry farms and tested for aerobic plate count (APC) to confirm for higher microbial presence (>4 $\log_{10}$ cfu/ml).

Trial 1: Four treatments were generated based on the differences in product treatments and/or dosing rates:

a: **Product A:** 0.33 LPM gas was continuously diffused into 1 liter (L) volume of microbial rich water (>4 $\log_{10}$ cfu/ml) during a 24 h trial period. This continuously treated water served as the test solution for Product A
b: **Product B:** Two stock solutions at two different concentrations were prepared by mixing product with deionized water

Stock 1: Lower dosing rate (LDR), where 2 ml of the product was mixed with 128 ml deionized water
Stock 2: Higher dosing rate (HDR), where 4 ml of the product was mixed with 128 ml deionized water

C: **Product C:** Stock solution was prepared by mixing 4 ml of the product with 128 ml deionized water

For Products B and C, the test solutions were made by adding 7.81 ml of stock solution to 1 L of the microbial rich water (>4 $\log_{10}$ cfu/ml) to make the test solutions. Each treatment had three replicates of test solution.

Trial 2: Four treatments were generated based on the differences in product treatments. Dosing rates for Product A and C were repeated as in trial 1. For Product B, the higher dosing rate of stock (4 ml of the product mixed with 128 deionized water) only was tested. A control of untreated microbial rich water (>4 $\log_{10}$ cfu/ml) solution was included in this test. Each treatment had three replicates of test solutions.

Microbial sampling: 5 ml volume of test solution per replicate was withdrawn in a sterile pipette during each sampling occasion for microbial plating. Each replicate of test solutions for all treatments was plated for APC and mold enumeration prior to the addition of product (0 h). Post addition of products, each solution was sampled at the following occasions:

1: **Trial 1:** 1/4, 1, 6, 18, 24 h
2: **Trial 2:** 1, 6, 12, 24 h

For both trials, test solutions remained covered to prevent access to sunlight, except during plating and residual measurement.

Microbial plating: Microbial plating was carried out for the test solutions sampled to enumerate APC and mold count using 3M Petrifilm™. One milliliter of water was directly plated on the Petrifilm and another milliliter was subjected to serial dilutions. Serial dilutions, up to a 3rd dilution level for APC and 2nd dilution level for mold, were performed by diluting one ml of drip sample in 9 ml of Buffered Phosphate Diluent (BPD) and then spinning the solution in the vortex mixture for 10 sec. At each dilution level, the plating was performed in duplicate to get the average microbial count. Enumeration of microbes was carried out after 48 h of incubation at 30°C for APC and after 72 h of sitting at room temperature (20°C) for yeast and molds.

Residual and pH measurement: Residuals for the products were measured during each microbial sampling for both trials. Water works test strips (Water Works™) were used to record the residuals and pH in the water.

Result analysis: All microbial counts were converted to $\log_{10}$ units prior to analysis to normalize data distribution. Results were then analyzed using GLM procedure of SAS. Significantly different means were separated using repeated t-tests at p-values less than 0.05.

RESULTS AND DISCUSSION

Residual results: The average residuals of different products evaluated for trial 1 and trial 2 over the different

Table 1: Drinking water quality standards for poultry (cfu/ml)

Source	Good	Maximum acceptable	Unacceptable
Main water supply	<100	<300	>300
Total aerobic plate counts	0	<1000	>1000
Total coliforms	0	50	>50
Fecal coliforms	0	0	1
E. coli	0	0	1
Pseudomonas	0	0	1

Table 2: Trial 1: Aerobic plate count (APC) before (0 h) and after (1/4, 1, 6, 18 and 24 h) treatment in $\log_{10}$ cfu/ml

Hours	Product A	Product B (LDR)	Product B (HDR)	Product C
0	4.59^{b}	4.45^{b}	5.17^{a}	4.53^{b}
0.25	5.09^{b}	4.61^{b}	4.46^{a,b}	4.53^{b}
1	4.7^{b}	4.98^{b}	3.49^{c}	4.19^{b}
6	4.53^{b}	4.23^{b}	4.29^{c}	3.54^{c}
18	1.60^{d}	3.74^{b,c}	3.84^{c}	3.52^{c}
24	1.91^{d}	3.81^{b,c}	3.77^{c}	3.19^{c}

^{a,b,c,d}Values with no common superscript in a row and column differ significantly

Table 3: Trial 1: Mold counts before (0 h) and after (1/4, 1, 6, 18 and 24 h) treatment given in $\log_{10}$ cfu/ml

Hours	Product A	Product B (LDR)	Product B (HDR)	Product C
0	2.58^{a}	2.28^{a}	2.37^{a}	2.27^{a}
0.25	1.74a	1.94^{a}	2.62^{a}	2.28^{a}
1	2.49^{a}	2.14^{a}	3.05^{a}	1.85^{a}
6	1.53^{a}	2.35^{a}	2.07^{a}	1.54^{a}
18	0.71^{b}	1.96^{a}	2.02^{a}	0.25^{b}
24	0.56^{b}	1.95^{a}	0.30^{b}	0.10^{b}

^{a,b}Values with no common superscript in a row and column differ significantly

Table 4: Trial 2: Aerobic Plate Count (APC) before (0 h) and after (1, 6, 12 and 24 h) treatment given in $\log_{10}$ cfu/ml

Hours	Product A	Product B	Product C	Control
0	4.71^{a}	4.7^{a}	4.81^{a}	4.52^{a}
1	2.11^{b}	2.93^{b}	2.69^{b}	4.26^{a}
6	1.33^{d}	2.66^{c}	2.14^{c}	4.23^{a}
12	1.19^{d}	2.61^{c}	1.68^{d}	4.1^{a}
24	0.66^{ef}	2.52^{c}	1.49^{d}	4.16^{b,a}

^{a,b,c,d,e,f}Values with no common superscript in a row and column differ significantly

Table 5: Trial 2: Mold counts before (0 h) and after (1, 6, 12 and 24 h) treatment given in $\log_{10}$ cfu/ml

Hours	Product A	Product B	Product C	Control
0	1.10^{b}	1.39^{b}	1.69^{b}	1.35^{b}
1	0.35^{d}	0.86^{cd}	1.43^{bc}	1.58^{b}
6	0.25^{d}	0.70^{c}	0.49^{d}	1.64^{b}
12	0.38^{d}	0.66^{c}	0.35^{d}	1.51^{b}
24	0.15^{d}	0.54^{c}	0.30^{d}	1.88^{a}

^{a,b,c,d}Values with no common superscript in a row and column differ significantly

sampling occasions are presented in line Figure: Fig. 1 and 2 (Product A), Fig. 3 and 4 (Product B) and Fig. 5 and 6 (Product C).

Product A produced a trace amount of hydrogen peroxide residuals in the test water. In both trials, Product A residual concentrations in the test water started off with ≤0.05 ppm, where it remained steady at 0.05 ppm throughout the trial period. The residual concentration of

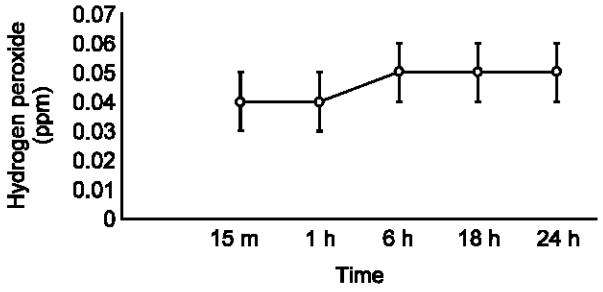

Fig. 1: Average residual trend, product A

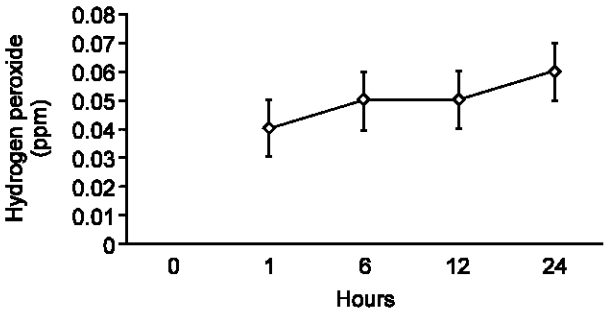

Fig. 2: Average residual trend, product A

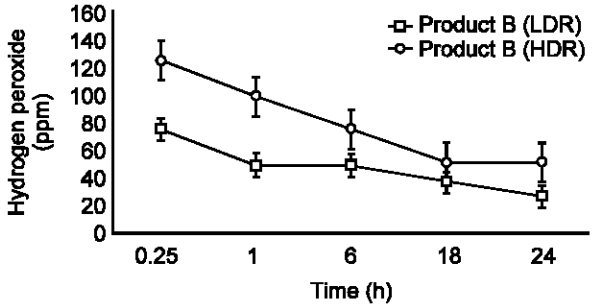

Fig. 3: Average residual trend, product B

product B in the test solution showed that there was no reduction in residuals below effective residual concentration (ERC) of 25 ppm in both the trials, even at LDR in trial 1. But the residual concentration of product B at HDR, in trial 1 was above 100 ppm hydrogen peroxide and remained above the ERC upper limit of 50 ppm for 24 h. The peroxide residual in trial 2 at 24 h was below 50 ppm. Product B at LDR in trial 1 was initially more than 50 ppm which dropped steadily over time but remained within the ERC throughout the 24 h trial period.

For Product C in trial 1, the initial residual concentration was below 5 ppm, above 5 ppm in trial 2 and continued to show a steady decline in residual concentration in both trials. Post 24 h treatment, the residual concentration was ≤1 ppm in trial 1 and slightly above 1 ppm in trial 2. pH recorded in the test solutions for all treatment groups throughout the trial period ranged at 7±0.2.

Microbial results:

Trial 1 results: The results of aerobic plate and mold counts at different sampling occasions for all treatment groups for trial 1 are presented in Table 2 and 3. In trial 1, all products started off with values of more than 4 log_{10} cfu/ml in test water. Product A showed a significant ($p<0.05$) reduction in the microbial count at 18 h post treatment and the bacterial count was reduced to 1.60 log_{10} cfu/ml. Product B at LDR showed no significant microbial reduction throughout the trial period, whereas at HDR, the significant decrease ($p<0.05$) of counts to 3.49 log_{10} cfu/ml was observed at 1 hour post treatment. The values remained fairly steady until 24 h and did not reduce to acceptable levels (<3 log_{10} cfu/ml). Product C showed similar trends as product B at HDR, where microbial counts were never reduced below 3 log_{10} cfu/ml.

Mold reductions were found to be significant ($p<0.05$) for product A and C after 18 h post treatment and 24 h post treatment for product B at HDR. Whereas, Product B at LDR did not show a significant reduction ($p>0.05$) in mold counts throughout the trial period.

Trial 2 results: The results of aerobic plate and mold counts at different sampling occasions for all treatment groups for trial 2 are presented in Table 4 and 5.

All the products A, B and C including the control started off with values of more than four and half log_{10} cfu/ml. Product A, Product B (at HDR) and Product C except for the control showed significant ($p<0.05$) reduction in microbial counts at 1 hour post treatment and all products were effective at reducing microbial populations within the acceptable range (<3 log_{10} cfu/ml). Mold reductions ($p<0.05$) were observed after 1 hour of treatment for Product A and Product B and 6 hours post treatment for Product C. The mold population remained steady throughout the trial period for the control. The mold population had higher (>2 log_{10} cfu/ml) counts in trial 1 than in trial 2 (<2 log_{10} cfu/ml). In both trials, none of the products tested completely eliminated the microbes within the 24 h trial period.

The goal of water sanitation procedures and sanitizer/disinfectant products is to target microbial challenges that exist and thrive in water supplies. Chlorine products, most commonly sodium hypochlorite or calcium hypochlorite, have been the primary water disinfectant products for several decades in the poultry industry. When drinking water has 2-5 ppm free chlorine residual, it is effective against most microbial growth in water (Watkins, 2007). However, chemical disinfection products using chemicals such as chlorine are not always used properly and thus, microbes are showing increased acquired resistance (Ridgway and Olson, 1982; Russell, 1999). Furthermore, the effect of chlorine as a water sanitizer is significantly reduced at weaker concentrations (Payment, 1999; Stern *et al*., 2002), in high pH of water (Galal-Gorchev, 1996; Park *et al*., 2004) and in water systems with well-established biofilms (De Beer, 1994).

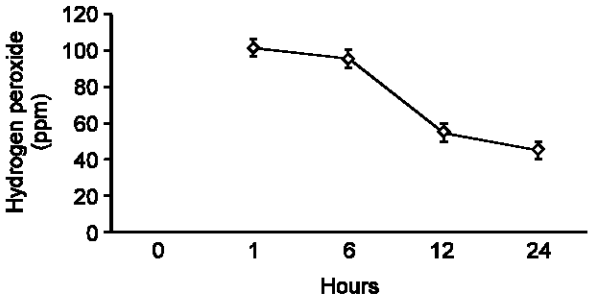

Fig. 4: Average residual trend, product B

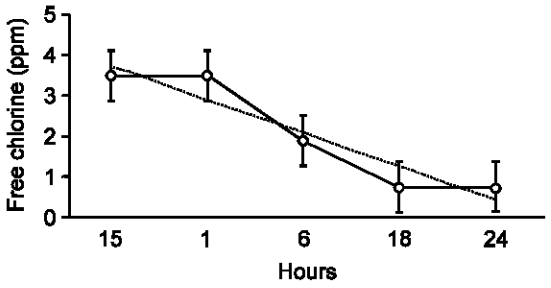

Fig. 5: Average residual trend, product C

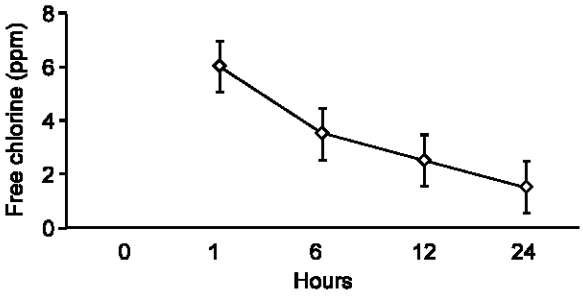

Fig. 6: Average residual trend, product C

There are farms that practice water sanitation programs utilizing hydrogen peroxide (H_2O_2) products (Agrisolutions, 2010) as an alternative to chlorine based sanitizers. Maintaining 25-50 ppm of hydrogen peroxide residuals in the water is considered as the Effective Residual Concentration (ERC) (Watkins, 2009). The industry/grower practices the use of the products, regardless of whether chlorine or peroxide based, without actually monitoring the residuals. This might compromise the effectiveness of the water sanitation procedures.

The AOP method of water treatment is an aqueous phase oxidation based method of destroying organic and some inorganic pollutants where highly reactive species such as hydroxyl radicals (primarily but not exclusively) are involved. Hydroxyl radicals have a strong

oxidation potential of 2.05 mV as compared to other oxidants such as Chlorine (1 mV) and hydrogen peroxide (Techcommentary, 1996; Carey, 1992). The results of these *in vitro* trials showed the comparative benefits of the AOP method of water treatment versus other products, demonstrating its possibility as an alternative water treatment method over conventional chemical methods. Field evaluations would further confirm the benefits of the AOP method.

To summarize an important observation made in this evaluation, for all products tested, Trial 1 required a higher residual contact time to reduce the bacterial counts significantly from its initial counts compared to Trial 2. The explanation for this difference in residual contact time for bacterial reduction may be attributed to higher mold levels in Trial 1. Therefore, the product efficacy in bacterial reductions could be impacted by mold populations in water.

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