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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Evaluating Different Hydrogen Peroxide Products for Residuals and Efficacy over Time

P. Maharjan, T. Clark, M. Frank, B.B. Martins, M.K. Foy and S. Watkins
Department of Poultry Science, Center of Excellence for Poultry Science,
University of Arkansas, Division of Agriculture, Fayetteville, AR-72701, USA

Abstract: Four commercially available hydrogen peroxide products were tested for residuals and efficacy over time. Each product was added at the rate of 59.14, 118.28 and 177.42 ml per 3780 ml of water creating stock solutions. Test solutions that actually mimic the bird drinking rate were made from each stock solution mixing at the rate of 29.57 ml of stock solution added to 3780 ml of water. Residual activities of test solutions prepared were measured from day 0 to day 5. Forty-eight hours post treatment, a 5 ml aliquot of water with a heavy microbial load was introduced into the test solutions as challenge and microbial plating for aerobic bacteria and mold was done for zero and one hour contact times. Results of this experiment suggest that an Effective Residual Concentration (ERC) of 25-50 ppm of hydrogen peroxide in test solution starts at 59. 14 ml of stock solution prepared for all products evaluated. Stabilized products stay at the higher residual level and can maintain ERC for a longer time than non-stabilized products. Significant bacterial reductions ($p<0.05$) within an hour of contact time was achieved at the lowest concentration tested, 59.14 ml of stock solution made, for all products provided that the ERC was maintained. Higher residuals or longer contact time were required for mold control.

Key words: Hydrogen peroxide, residuals, water, efficacy

INTRODUCTION

The poultry industry understands the value of clean and sanitized water supplies for optimizing bird performance and reducing the costs associated with grow-out. Disinfecting water with chlorine for human drinking purpose has been a century old practice in the US (McGuire, 2006) and is considered as the standard practice of water sanitation in animal husbandry as well. Nevertheless, the use of chlorine sanitizer in a high pH of water (Galal-Gorchev, 1996; Park *et al*., 2004), or at weaker concentrations (Payment, 1999; Stern *et al*., 2002), or when the water systems have established biofilms (De Beer *et al*., 1994), results in a significant reduction in the sanitizing efficacy of chlorine. In commercial production barns, newly hatched chicks and poults are provided water supplies that are warmed to prevent chilling the 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 small volume of water usage means water often remains in waterlines for several hours. This results in loss of efficacious chlorine residuals which could leave birds vulnerable to microbial challenges from biofilms. It is of high interest to the industry to identify alternative water sanitizers which could remain efficacious for extended periods of time.

Recent field experiences have shown that poor performing poultry farms are greatly benefitted from a water sanitation program utilizing hydrogen peroxide (H_2O_2) products (Agri-solultions, 2010). Maintaining 25-50 ppm of hydrogen peroxide residuals in the water is considered as the Effective Residual Concentration (ERC) (Watkins, 2009). There are numerous sources of H_2O_2 products available for poultry water system sanitation and their concentration ranges from 20-50% with or without stabilizers. The industry/grower practices the use of those products without actually monitoring the residuals.

Therefore, this study was conducted with the objective of determining baseline information on different H_2O_2 products prepared at different concentration levels for residual activities over time. To measure how effective these solutions were in limiting or reducing microbial growth when challenged with heavy laden microbial water was the second study goal.

MATERIALS AND METHODS

An *in vitro* experiment was carried out to evaluate different hydrogen peroxide products for residuals and efficacy over time. In the experiment, two trials, trial 1 and trial 2, were conducted following similar methods, trial 2 being the repeated test for trial 1. Any procedures carried out in trial 2 differently than in trial 1 were reported in

Corresponding Author: P. Maharjan, Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Division of Agriculture, Fayetteville, AR-72701, USA

methods, otherwise would imply the same methods executed for both the trials.

Hydrogen peroxide products: Four commercially available hydrogen peroxide products commonly used in poultry drinking water disinfection system were obtained for evaluation:

1: Product A-50% H_2O_2 with silver complex
2: Product B-20% H_2O_2 with peracetic acid mixture
3: Product C-34% H_2O_2
4: Product D-28% H_2O_2

Products A, B and C were stabilized whereas product D was not.

Water used: Municipal water was used for preparing the stock and test solutions for both the trials. Before the water was used for preparing the solutions, it was allowed to sit for 48 h in open container to dissipate the chlorine residual.

Preparation of stock and test solutions: Each product was added at the rate of two, four and six ounces (59.14, 118.28 and 177.42 ml per gallon (3780 ml) of water creating stock solutions and then final mixtures as test solutions were made from each stock solution by mixing an ounce (29.57 ml) of stock solution added to a gallon of water. For this *in vitro* evaluation, one ml of each stock solution prepared was pulled and added to 128 ml of water. These test solutions actually mimic the medicator injection rate of 1:128 that is commonly used for adding water products to the drinking water. Each test solution and the control without any treatment were replicated thrice for both the trials. After the solutions were prepared they were covered to prevent sunlight access, except during the residual measurement and microbial plating.

Residual measurement: Peroxide residuals were measured for each test solution from day 0 to day 5 in both the trials. In trial 1, the residual measurement was carried out using Water Works test strips that measure from less than 0.5 ppm to 100 ppm. In trial 2, Mini Analyst Series 942 Hydrogen Peroxide meter was used that can measure up to 100 ppm of peroxide residual activities providing precise numerical values.

Challenge introduction and microbial plating: At 48 h post treatment, a 5ml aliquot of microbial water was added as challenge was introduced to two replicates of each of the treatments and two replicates of control. A third replicate of each treatment and control were kept challenge free. Microbial plating were then carried out for aerobic plate count (APC) and mold count at 0 h (immediately after challenge introduction) and 1 h post challenge introduction using Petrifilm™. In trial 2, 24 h post treatment plating was also added. Enumeration of microbes in colony forming units (cfu) 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 molds.

Result analysis: All microbial counts were converted to $\log_{10}$ units prior to analysis to normalize data distribution. Results were then analyzed using JMP Pro10 software using one way ANOVA (JMP Pro, 2012). Means were considered significantly different for $p<0.05$.

RESULTS AND DISCUSSION

Residual results: The average residual activities of different hydrogen peroxide products for trial 1 and trial 2 over days are presented in Table 1 and 2, respectively. In both the trials, Product A maintained a higher peroxide residual level followed by product C while product D remained the lowest among all 4 products at each concentration level from day 0 to 5. However, product D at the 2 ounces stock solution concentration level maintained the lower limit of ERC of 25 ppm in test solution until day 1 in trial 1 and until day 2 in trial 2. The residual activity of product D in test solution was significantly lower ($p<0.05$) than all other test solutions prepared from stabilized products A, B and C when it started to drop off below the ERC at this concentration level of 2 ounces. Other stabilized products A, B and C at the 2 ounces stock solution concentration level maintained ERC in test solutions at least a day more than non-stabilized product D. In trial 2, at 4 and 6 ounces stock solution concentration levels, stabilized products A, B and C were above the ERC in test solutions all days throughout the trial period. Even the non- stabilized product, D, maintained the peroxide residual above the ERC in test solution at 6 ounces concentration level until day 5.

In both the trials, reduced residual activities were noticed in test solutions that were challenge introduced than those without challenge for all products and all concentration levels.

Microbial results

Trial 1: The results of aerobic plate count and mold count at 0 and 1 h post challenge introduction in test solutions for trial 1 are presented in Table 3 and 4.

Immediately after the challenge introduction (at 0 h contact time) on day 2, there were significant reductions in bacterial count ($p<0.05$) with all the products at all concentration levels as compared to the control (>4 $\log_{10}$ cfu/ml for control vs <3 $\log_{10}$ cfu/ml for all products). At the 1 h post inoculation interval, there was again a reduction by a log with respect to the count values observed at the 0 h contact time for all the products and at all concentration levels. An important thing to notice was there were no significant differences in bacterial reduction within the product at 2, 4 and 6 ounces

Table 1: Trial 1 Average residual activity (in ppm) of different dydrogen peroxide products over a 5 day period

Concentration (ounces/gallon)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Product A, 2	>100^{a}	>50^{d}	>50^{d}	25^{h}	<25^{j}	>10^{l}
Product B, 2	50^{e}	<50^{f}	<25^{i}	10^{k}	<10^{l}	>5^{m}
Product C, 2	50^{e}	<50^{f}	25^{h}	>10^{j}	<10^{l}	<10^{l}
Product D, 2	50^{e}	25^{h}	<25^{i}	<10^{l}	>5^{m}	<5^{o}
Product A, 4	>100^{a}	>100^{a}	100^{b}	50^{e}	<50^{f}	>25^{g}
Product B, 4	>100^{a}	<100^{c}	50^{e}	25^{h}	<25^{i}	<25^{i}
Product C, 4	<100^{c}	50^{e}	<50^{f}	>25^{g}	>25^{g}	25^{h}
Product D, 4	<100^{c}	50^{e}	<50^{f}	25^{h}	>10^{j}	>10^{l}
Product A, 6	>100^{a}	>100^{a}	>100^{a}	>100^{a}	100^{b}	<100^{c}
Product B, 6	>100^{a}	>100^{a}	100^{b}	>50^{d}	<50^{f}	>50^{d}
Product C, 6	>100^{a}	100^{b}	<100^{c}	>50^{d}	50^{e}	<50^{f}
Product D, 6	>100^{a}	<100^{c}	50^{e}	>25^{g}	>25^{g}	>25^{g}

$^{a-o}$Means with different superscripts are significantly different ($p<0.05$)

Table 2: Trial 2 Average residual activity (in ppm) of different hydrogen peroxide products over a 5 day period

Concentration (ounces/gallon)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Product A, 2	79.0^{e}	76.7^{e}	64.2^{gh}	58.6^{hijk}	55.5^{klm}	>50^{lmn}
Product B, 2	44.4^{op}	37.1^{pq}	32.9^{s}	27.0^{tu}	26.3^{u}	>10^{w}
Product C, 2	53.5^{klm}	49.6^{mn}	41.2^{pqr}	36.5^{qrs}	32.6st	>10^{w}
Product D, 2	36.3^{rs}	34.1^{s}	26.6^{tu}	22.1^{uv}	19.2^{v}	>10^{w}
Product A, 4	>100^{a}	>100^{a}	>100^{a}	100.5^{a}	98.7^{ab}	<100^{ab}
Product B, 4	83.1^{e}	77.2^{e}	67.5^{fg}	58.8^{hijk}	57.6^{ijkl}	>50^{lmn}
Product C, 4	98.3^{ab}	94.9^{bc}	77.6^{e}	67.6^{fg}	63.1^{ghi}	50.0^{mno}
Product D, 4	70.2^{f}	70.4^{fg}	55.8^{jkl}	45.2^{nop}	45.1^{nop}	<50^{mno}
Product A, 6	>100^{a}	>100^{a}	>100 ^{a}	>100^{a}	>100^{a}	>100^{a}
Product B, 6	>100^{a}	>100^{a}	97.5^{ab}	88.0^{d}	88.0^{d}	<100^{ab}
Product C, 6	>100^{a}	>100^{a}	>100^{a}	>100^{a}	98.2^{abc}	<100^{ab}
Product D, 6	99.7^{ab}	93.2^{cd}	76.7^{e}	60.8^{hij}	57.8^{ijk}	>50^{lmn}

$^{a-w}$Means with different superscripts are significantly different ($p<0.05$)

Table 3: Trial 1 Aerobic plate count (log_{10} cfu/ml) at 0 and 1 h post challenge introduction

Concentration (ounces/gallon)	0 h	1 h
Product A, 2	3.84^{bc}	2.61^{g}
Product B, 2	3.52^{de}	2.62^{g}
Product C, 2	3.72^{cd}	2.61^{g}
Product D, 2	3.72^{bcd}	2.71^{g}
Product A, 4	3.53^{de}	2.63^{g}
Product B, 4	3.23^{f}	2.72^{g}
Product C, 4	3.90^{bc}	2.66^{g}
Product D, 4	3.74b^{cd}	2.65^{g}
Product A, 6	3.45^{ef}	2.63^{g}
Product B, 6	3.29^{ef}	2.74^{g}
Product C, 6	3.71^{cd}	2.56^{g}
Product D, 6	3.71^{cd}	2.76^{g}
Control	4.22^{a}	3.97^{b}

$^{a-g}$Means with different superscripts differ significantly ($p<0.05$)

concentration levels for all products at both 0 and 1 h contact time although there were significant differences in their residual activities in these levels. Mold reductions were found to be significant ($p<0.05$) only at 6 ounces concentration level by an hour of contact time for all products.

Trial 2: The results of aerobic plate counts and mold counts at 0 and 1 h post challenge introduction in test solutions for trial 2 are presented in Table 5 and 6.

Trial 2 had a higher starting off bacterial counts (> 5 log_{10} cfu/ml) presenting even a greater challenge condition than trial 1. Only product B at all concentration levels (2, 4 and 6 ounces) had significant reductions ($p<0.05$) in bacterial counts at 0 hour contact time than the control. However, by one hour of contact time, all products at all concentration levels dropped the bacterial count to a significantly lower ($p<0.05$) level as compared to the control. However, the counts values in test solutions for all the products except for product B were above 4 log_{10} cfu/ml. An important point to notice again was there were no significant differences in bacterial reduction in challenge introduced test solutions within the product at 2, 4 and 6 ounces concentration levels for all products at both the 0 and 1 h contact times although there were significant variation in residual activities at these levels. Mold counts were found to be significantly lower for stabilized products A, B and C only at 6 ounces concentration level by an hour of contact time.

In both the trials, none of the products at any concentration level tested completely eliminated the microbes by one hour of contact time.

Earlier studies conducted in different disciplines show hydrogen peroxide as an effective antimicrobial agent. Hydrogen peroxide (H_2O_2) has a strong oxidizing property for biomolecules and its oxidizing property and efficacy

Table 4: Trial 1 Mold count (log_{10} cfu/ml) at 0 and 1 h post challenge introduction

Concentration (ounces/gallon)	0 h	1 h
Product A, 2	1.00^{defg}	1.13^{abcdef}
Product B, 2	1.23^{ab}	1.21^{abc}
Product C, 2	1.09^{bcdef}	1.12^{abcdef}
Product D, 2	1.19^{abcd}	1.08^{bcdef}
Product A, 4	0.84^{ghi}	0.92^{fgh}
Product B, 4	1.15^{abcde}	1.15^{abcde}
Product C, 4	1.10^{bcdef}	1.02^{cdefg}
Product D, 4	1.04^{bcdefg}	0.95^{efgh}
Product A, 6	0.69^{ijk}	0.54^{k}
Product B, 6	1.02^{bcdefg}	0.59^{ijk}
Product C, 6	1.11^{abcdef}	0.69^{ijk}
Product D, 6	1.16^{abcde}	0.75^{hij}
Control	1.14^{abcde}	1.31^{a}

$^{a-k}$Means with different superscripts differ significantly ($p<0.05$)

Table 5: Trial 2 Aerobic plate count (log_{10} cfu/ml) at 0 and 1 h and 24 post challenge introduction

Concentration (ounces/gallon)	0 h	1 h	24 h
Product A, 2	5.60^{ab}	4.93^{efgh}	2.77^{lm}
Product B, 2	5.23^{cde}	3.21^{k}	2.77^{lm}
Product C, 2	5.27^{bcd}	5.09^{defg}	2.87^{lm}
Product D, 2	5.69^{a}	5.10^{defg}	3.04^{kl}
Product A, 4	5.56^{abc}	4.72^{hij}	2.87^{lm}
Product B, 4	5.12^{def}	2.69^{m}	2.65^{mn}
Product C, 4	5.74^{a}	4.81^{fghij}	2.77^{lm}
Product D, 4	5.59^{ab}	4.79^{ghij}	2.73^{lm}
Product A, 6	5.60^{ab}	4.49^{j}	2.76^{lm}
Product B, 6	4.52^{ij}	2.69^{m}	2.33^{n}
Product C, 6	5.69^{a}	4.73^{hij}	2.74^{lm}
Product D, 6	5.62^{a}	4.83^{fghi}	2.84^{lm}
Control	5.79^{a}	5.75^{a}	5.87^{a}

$^{a-n}$Means with different superscripts differ significantly ($p<0.05$)

Table 6: Trial 2 Mold Count (log_{10} cfu/ml) at 0 and 1 h and 24 post challenge introduction

Concentration (ounces/gallon)	0 h	1 h	24 h
Product A, 2	0.94^{abc}	0.87^{abcdef}	0.48^{ghi}
Product B, 2	0.92^{abcd}	0.92^{abcd}	0.35^{i}
Product C, 2	0.70^{cdefg}	0.90^{abcde}	0.81^{abcdef}
Product D, 2	0.95^{abc}	0.93^{abcd}	0.70^{cdefg}
Product A, 4	0.72^{cdefg}	0.74^{bcdefg}	0.00^{j}
Product B, 4	0.93^{abcd}	0.63^{fgh}	0.30^{hijk}
Product C, 4	0.90^{abcde}	0.95^{abc}	0.50^{ghi}
Product D, 4	1.00^{ab}	0.84^{abcdef}	0.66^{defg}
Product A, 6	0.69^{cdefg}	0.65^{efg}	0.00^{jk}
Product B, 6	0.65^{efg}	0.65^{efg}	0.00^{j}
Product C, 6	0.74^{bcdefg}	0.63^{fgh}	0.00^{j}
Product D, 6	0.85^{abcdef}	.91^{abcde}	0.30^{ijk}
Control	1.08^{a}	1.02^{a}	0.93^{abcd}

$^{a-k}$Means with different superscripts differ ($p<0.05$)

are greatly affected by the formulation and physical state (Finnegan *et al*., 2010). This disinfectant at 3% has a rapid bactericidal effect and is effective against a wide range of viruses, yeast and fungi (Block, 2001). Compounds like silver and peracetic acid in hydrogen peroxide have shown to synergize with the disinfecting property of hydrogen peroxide (Alasri *et al*., 1992; De Velasquez *et al*., 2008; Pedahzur *et al*., 1995; Pedahzur *et al*., 1997).

The use of various concentrations of hydrogen peroxide has been studied for their antimicrobial efficacies in both human and animal research. A solution 0.03% hydrogen peroxide proved effective in controlling *E. coli* and *Salmonella* load in fruit juices (Schurman, 2001). 2% hydrogen peroxide for 3 h contact time (Ruano *et al*., 2001) and 3% solution for one hour of contact time showed complete antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus fumigatus* and *Fusarium* species with organic matter present (Gehan, 2009). Furthermore, hydrogen peroxide acts as surface disinfectant and is effective against the biofilms such as of *Salmonella* and *Staphylococci* (Carrique-Mas *et al*., 2009; Marin *et al*., 2009; Payne *et al*., 2005). Peroxide based disinfectants also perform well in inactivating *Pseudomonas aeroginosa* (Wirtanen *et al*., 2001) and *Listeria monocytogenes* biofilms (Robbins *et al*., 2005).

In previous studies conducted at the University of Arkansas, different stabilized and non-stabilized hydrogen peroxide products were evaluated for residuals and efficacy over time and had similar results as in this study (Clark *et al*., 2009; Hancock *et al*., 2007). Based on these *in-vitro* evaluations, a few conclusions can be drawn. (1) Effective Residual Concentration (ERC) of hydrogen peroxide in drinking water starts at 2 ounces per gallon of stock solution for all the products evaluated. At this rate, non-stabilized product maintain ERC in drinking water for 2-3 days whereas stabilized products maintain longer (at least one day long) than non- stabilized. (2) One h of contact time is adequate to reduce the bacterial load significantly under the high challenge condition, provided that the ERC is maintained. Residual activity of hydrogen peroxide in water above the ERC (of 25-50 ppm) does not mean better bacterial control than when it is at ERC. Higher concentrations or longer contact time are required for mold control. Therefore, depending upon the nature of microbial problem in water, appropriate disinfection strategy should be utilized. (3) Disinfecting the water with these tested products at 4 and 6 ounces per gallon of water to make stock solutions leave higher residuals than ERC in test solutions for several days. Future studies can be carried out for the maximum tolerable residuals the chicks/birds can drink without health compromise.

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