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Treating Poultry Drinking Water for Waterline Biofilm Mitigation

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Water is vulnerable to contamination and biofilm buildup

In poultry production, water quality can be correlated with the body weight, feed conversion, livability and condemnation and thus it affects the overall performance of birds. Every farm should routinely test their water supplies for its microbial and mineral content to assure that these parameters are within the acceptable range of poultry drinking water standards. An evaluation of water supplies conducted in U of Arkansas, broiler research farm- Savoy unit, showed that water systems are vulnerable to microbial contamination to unacceptable microbial levels (> 3 log10 cfu/ml) regardless of consistent water sanitation and thus water systems are prone to biofilm built up over time.

Poultry waterlines are major portion of poultry water system and are generally constructed of polyvinylchloride (PVC) material. Several studies suggest that water systems with PVC pipe material can grow biofilm even when the water supply is clean, potable and treated. Non-sanitized water systems can harbor high levels of biofilm in water lines and foul the water supply. Biofilm are complex communities of different species of enclosed microbial cells cooperating with one another for survival and are firmly attached to hydrated surfaces. Biofilm bacteria are different from their free-living planktonic counterparts in terms of growth rate and composition and show increased level of resistance to disinfectants. Water system biofilm can harbor pathogens such as *Campylobacter*, *Salmonella*, *Escherichia coli* including avian pathogenic (APEC) strains, *Pseudomonas*; protozoans, and viruses. These organisms enter water system and incorporate into established biofilm, and thus enhance the risk of flock positivity to these pathogens. Birds, particularly chicks, remain vulnerable to microbial challenges from biofilm. Further, biofilm clogs water pipes and filters, and thus, restrict water flow which can lead to poor flock performance.

It would be worthwhile to understand the nature of biofilm growth in waterlines, especially during the first week of brooding when water supplies are warmed and have very slow flow. In addition, water quality typically supplied in commercial poultry houses can also contain nutrients such as iron and manganese which are required for growth of some pathogenic microbes. Therefore, *in vitro* experiments were designed to develop a model that would mimic the conditions of warm, slow moving water thus providing a way to monitor biofilm growth over time and to determine if this phase of poultry production would increase the susceptibility of water systems to biofilm development. PVC sections (internal surface area 15.16 cm^2) were utilized in the study to grow biofilm in slow moving warm test water. Test water was characterized for mineral and microbial content for each experiment replication. Water was considered sub optimal microbial quality (unacceptable for poultry) if the microbial enumeration was > 3 $\log_{10}$ cfu/ml. A primary objective of the study was to understand the differences in the biofilm growth rate on PVC surface when exposed to microbiologically acceptable poultry drinking water (< 3 $\log_{10}$ cfu/ml) versus sub optimal microbial water (> 3 $\log_{10}$ cfu/ml) under treated and untreated conditions.

Experiment 1. Biofilm growth in low microbial content water

This study was performed to determine if biofilm would develop when PVC test coupons were exposed to low microbial content warm water and also to determine if biofilm development would be influenced by adding a sanitizer. Table 1 and 2 gives the minerals and microbial parameters of water used in the study. Table 3 gives the residuals recorded in test water for the sanitizers used. Figure 1 and 2 gives the microbial results for test water and test coupons. This experiment showed that biofilm could develop in minimally contaminated water even in the presence of sanitizers, yet chlorine was more effective than hydrogen peroxide in limiting this development.

Table 1. Minerals characterization (in ppm) of test water*

	B	Mg	Si	Ca	P	S	Na	Cl	pH
Test Water	-	2.13	3.1	27	-	-	6.12	7.7	8.2

* Cr, Mn, Fe, Co, Ni, Cu Zn, As, Se, Mo, Cd, Sb, Be, Ba, Al and Pb were measured to be either <0.03 ppm or non-detectable (N. D.) in both the test waters

Table 2. Microbiological and other parameters of test water

	APC (cfu/ml)	Total coliforms	Conductivity (µS/cm)	*TOC (ppm)
Test Water	1000	0	191	1.28

*TOC is total organic carbon.

Table 3. Average residuals recorded in test solutions[1]

	Day 1	Day 4		day 7		day 10
	after dosing product	during sampling	after dosing product	during sampling	after dosing product	during sampling
CBP	2- 3	< 0.25	2- 3	< 0.5	2 - 3	< 0.25
HPB	> 50ppm	15 - 25	> 50ppm	10-25	> 50ppm	10-25

[1] Residuals measured in ppm, n= 3
[2]CBP: Chlorine based product, 8.25 % sodium hypochlorite; [3]HPB: Hydrogen peroxide-based product, 30 % concentrate; Products were dosed in test water at every 72 hours during the study period. Stock solutions for the products were initially prepared mixing 1 ml of the product to 32 ml of deionized water, and then added to test solution at the ratio of 1 ml stock to 128 ml of test water.

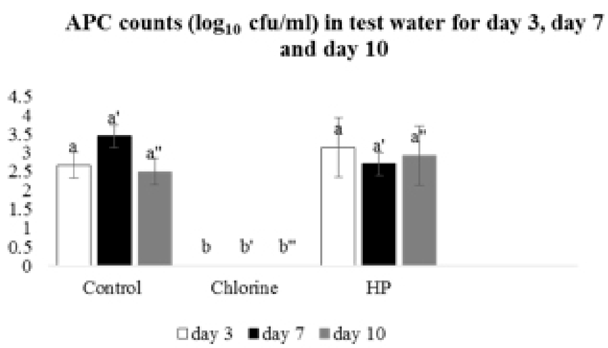

Figure 1. Test water was sampled on day 3, day 7 and day 10. Samples were plated for APC. Treatments were compared for sampled days. Different letters on the top of bars for sampled days are significantly different. Control = no sanitizer; Chlorine = Chlorine based product; HP=Hydrogen peroxide based product

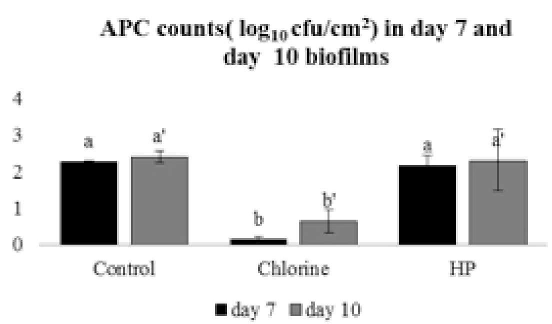

Figure 2. Test coupons were sampled on day 3, day 7 and day 10. Biofilms were swabbed and were plated for APC. Treatments were compared for sampled days. Different letters on the top of bars for sampled days are significantly different. Control = no sanitizer, Chlorine = Chlorine based product; HP=Hydrogen peroxide based product.

Experiment 2. Biofilm growth in sub- optimal microbial quality of water

How quickly biofilm grow in waterlines if the water supply has sub-optimal microbial quality can be easily overlooked. Similar study as Experiment 1 was conducted but using sub optimal water (> 3 $\log_{10}$ cfu/ml) and the effect of sanitizers was tested to know how effectively it can lower the microbial content of test water, and also to understand its efficacy to inhibit the biofilm growth in the test coupons. Table 4 gives the water quality characteristics of test water used in the study. Table 5 gives the residual concentration during the sampling occasion. Figure 3 and 4 give the microbial results for test water and test coupons. Results showed that treating water help reduced the microbial counts in suboptimal water significantly to acceptable level of poultry drinking water standard. However, biofilm can still develop in contaminated water even in the presence of sanitizers. Untreated water developed higher level of bacterial growth of > 4 $\log_{10}$ cfu/cm^2 by 48 hours in test coupons.

Table 4. Minerals characterization (in ppm) of test water*

	Fe	Mg	Mn	Ca	P	S	Na	Cl	pH
Test Water	0.05	3.29	0.02	65.43	<5	4.72	<5	0.01	7.57

* Cr, Co, Ni, Zn, Mo, Cd, Al and Pb were measured to be either <0.03 ppm or N. D. in both the test water

Table 5: Residual concentration measured in test solutions treated with HPBP and CBP over time during sampling occasions[1]

Post sanitizer application (hours)	[2]HPBP (hydrogen peroxide in ppm)	[3]CPB (Free chlorine in ppm)
0	>50	~2.5
1	≥ 50	1-2.5
6	<50	1
24	~ 30	<1
48	< 30	0.1
72	10 to 30	0.1

[1] Residuals measured in ppm, n= 3

[2]HPBP: Hydrogen peroxide based product; [3]CBP: Chlorine based product; Dosing rates of the products was carried out similarly as Experiment 1.

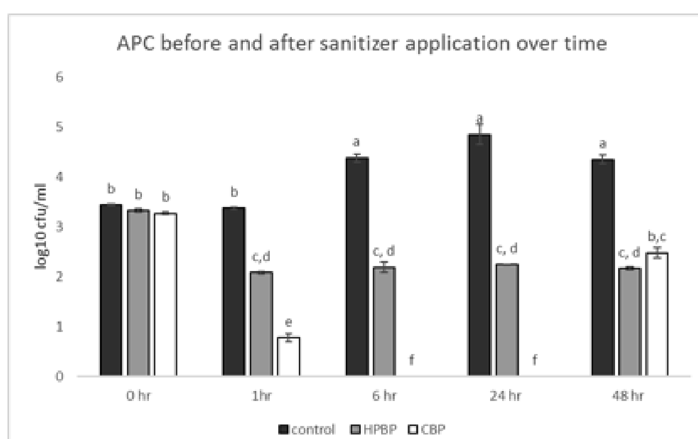

Figure 3. Test water was sampled for APC at 0 hour (before treatment), 1 hour, 6 hours, 24 hours and 48 hours post sanitizers treatment and were plated for APC. Treatments were compared for sampled

occasions. Different letters on the top of bars for sampled days are significantly different. Control = no sanitizer; HPBP= Hydrogen peroxide based product; CBP = Chlorine based product.

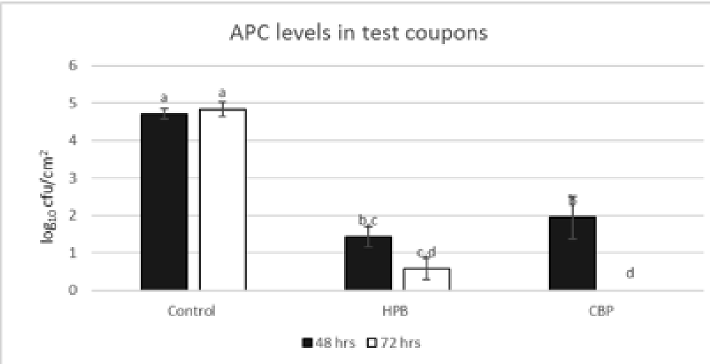

Figure 4. Test coupon were sampled at 48 hours and 72 hours post sanitizer application. Biofilm present in test coupons were swabbed and were plated for APC. Treatments were compared for sampled occasions. Different letters on the top of bars for sampled days are significantly different. Control = no sanitizer; HPB= Hydrogen peroxide based product; CBP = Chlorine based product.

Experiment 3. Sanitizers effect on already formed biofilm

Two trials were conducted in this experiment. First, biofilms were grown in PVC test coupons for the period of 7 days and using sub optimal microbial warm water (in ◦C, from d 2 to d 6, temperature of test water was maintained at 31.6, 31.1, 30.5, 30, and 29.4 ◦C, respectively), and then the efficacy of sanitizers were tested for the removal of 7-d old biofilm formed. Table 6 and 7 give the water quality parameters for the test water used in the trials. Residuals results noted are presented in table 8, and microbial results for biofilm removal post sanitizer treatment is given in Figure 5. Results of this study indicate that bacterial biofilm formation (> 3.5 $\log_{10}$ cfu/cm²) can occur quickly (≤ 7 d) in poultry waterlines under warm water temperature condition and when water supplies are sub optimal microbial quality. However, use of sanitizers such as chlorine or hydrogen peroxide based sanitizers can help mitigate already formed biofilm.

Table 6. Minerals characterization (in ppm) of test water*

	Ba	B	Mg	Se	Ca	Zn	P	Mn	S	Na	Cl	pH
Test Water 1	0.04	0.04	2.64	0.03	28.7	N.D.	1.38	N.D.	1.10	3.75	7.82	7.25
Test Water 2	0.02	0.74	3.97	N.D	23.3	0.13	N.D	0.03	1.20	75.3	35.9	7.98

* Cr, Fe, Co, Ni, Cu, As, Mo, Cd, Sb, Be, Ba, Al and Pb were measured to be N. D. in both the test waters

Table 7. Microbiological and other parameters of test water

	APC	N (Nitrate + Nitrite)	TOC (ppm)
Test Water 1	5.08	4.15	4.48
Test Water 2	5.25	3.02	2.32

*TOC is total organic carbon; N = Nitrogen

Table 8. Average residuals recorded in test solutions[1]

	[4]Immediately		Post 24 hours		Post 48 hours	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
CBP[2]	4 - 5	2-3	3-4	2	2 - 3	>1
HPB[3]	50-100	>50	>50	25-50	25-50	>25

[1] Residuals measured in replicates (n= 3) measured the same for both the treatments. [2]CBP: Chlorine based product; [3]HPB: Hydrogen peroxide based product; [4]Post 0 hour is the residual measurement immediately after sanitizer application in test water; Dosing rate of the products were carried out similarly as discussed in the Experiment 1.

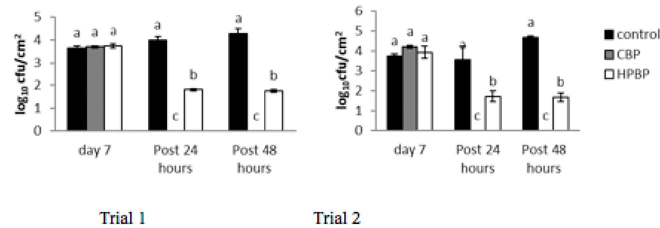

Figure 5. Trial 1 and 2: Bacterial biofilm levels observed on PVC test coupons when exposed to suboptimal microbial water (> 4 $\log_{10}$ cfu/ml) for 7 days. Post 7 days, test coupons that had 7 d old biofilms were transferred to bacteria free water and treated with sanitizers. Biofilm levels were measured post 24 and 48 hours sanitizer treatment. CBP: Chlorine Based Product; HPBP: Hydrogen Peroxide Based Product. [a,b,c] Different letters on top of the bars are significantly different ($P < 0.05$).

Summary:

- These studies demonstrate that regardless of clean and treated water supply, water system is susceptible to biofilm growth especially when barn temperature is warm at early grow- out period. Biofilm growth more than 4 $\log_{10}$ cfu/cm² can occur quickly (< 7 days) in waterlines if water is not treated and water supply is sub optimal (> 3 $\log_{10}$ cfu/ml) type.
- This evaluation also shows that treating water either with chlorine or hydrogen peroxide based sanitizer can be an effective water sanitation measures to address microbial problem in water or to mitigate biofilm related issues in water system.
- Therefore, water supplies require daily and uninterrupted treatment especially at early grow out period to maintain microbiologically safe water for chicks and to keep system hygiene clean.

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*Details on study methods for the results presented are not discussed in the article. However, any queries regarding the study details can be directed to authors at pmaharja@uark.edu or swatkin@uark.edu

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