ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 12 (9): 505-508, 2013
ISSN 1682-8356

Effects of Bac-D on Total Aerobic Bacteria Naturally Found on Broiler Breeder Eggs

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Abstract: Hatchery sanitation is of the utmost importance in the poultry industry and may have drastic economic effects within a company. It has been shown that eggs with increased total aerobic bacterial counts may cause a decrease in hatchability, performance and growth, as well as a decrease in overall chick quality. Several methods have been utilized to decrease bacterial load on the exterior surface of the egg such as the use of: hydrogen peroxide, quaternary ammonium compounds, antibiotics and UV light exposure. Many disinfectants may effectively sanitize the egg; however, they have detrimental effects on the developing embryo due to the removal of the cuticle, allowing increased moisture loss from the egg. Benzalkonium chloride has been effectively used as a first aid antiseptic for humans. Bac-D, a novel disinfectant with benzalkonium chloride utilized as the active ingredient. Bac-D is a safe, potential substitute to harsh chemicals. In this trial, eggs were sprayed with the same volume of either Bac-D or water. Eggs were sampled at 3 different time points after spray (0, 1.5, or 3 h). At the culmination of each time point, a portion of the eggs was inoculated with an endogenous bacterial inoculum. Eggs were placed in a bag with 1% PBS and the rinsate was promptly plated on TSA (Tryptic Soy Agar). There were significant decreases ($p<0.0001$) in the log CFU/mL numbers at each time point (0, 1.5, 3 h). These results reveal the potential sanitizing effects of Bac-D on total aerobic bacterial counts on eggs.

Key words: Eggs, egg wash, disinfectant, total aerobic bacteria, Bac-D

INTRODUCTION

Hatchery sanitation is a crucial factor in today's poultry industry. Since poultry are produced in such large numbers, even very small deviations can have drastic economical impacts. Eggs with increased total aerobic bacterial counts can cause a decrease in hatchability, performance and growth, as well as a decrease in chick quality (Coufal *et al*., 2003). Many different techniques have been explored to determine the correlation between reducing bacteria present on eggs and improving hatchability.

Some of the methods used to reduce bacteria on the shell of the egg include: ethylene oxide (Lorenz *et al*., 1950), hydrogen peroxide (Sheldon and Brake, 1990), quaternary ammonium sanitizers (Brake and Sheldon, 1989), antibiotics (Miller, 1956), UV light exposure (Coufal *et al*., 2003) and heat treatment (Funk *et al*., 1954). Many disinfectants may effectively sanitize eggs; however, they have detrimental effects on the developing embryo. This phenomenon occurs because sanitizers may remove the cuticle, a protective thin layer that seals the egg, which allows for an increase in loss of moisture from the egg, which directly affects the embryo (Peebles and Brake, 1985).

Currently, formaldehyde fumigation is the most commonly used intervention method for egg sanitation in the industry (Coufal *et al*., 2003). The main disadvantages associated with the use of formaldehyde are its carcinogenic properties as well as its effect on decreasing hatchability (Fasenko *et al*., 2009). As a result, there has been much effort aimed at finding alternative approaches to the use of formaldehyde (Brake and Sheldon, 1989).

Since rate of hatchability is a major consideration when searching for alternative hatching egg disinfectants, it may be considered as a valuable parameter to measure the efficacy of different disinfecting compounds. The use of UV light as a sanitation procedure compared to water resulted in no significant differences in hatchability when compared to untreated eggs (Coufal *et al*., 2003). Broiler eggs fogged with ozonated water for 30 min caused significantly reduced hatchability rates, while eggs fogged with triple strength formaldehyde remained unaffected (Whistler and Sheldon, 1988). Electrolyzed water treated eggs showed similar hatching rates between the treatment and control groups (Fasenko *et al*., 2009). All approaches herein mentioned have shown mixed hatchability rates thereby eliciting the increasing need for a method, which has consistent and effective results.

Benzalkonium chloride, a quaternary ammonium compound, generally recognized as safe, has been effectively used as a first aid antiseptic for humans through the years (Dyer *et al*., 1998; Gerald *et al*., 2011).

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The mode of action of benzalkonium chloride consists on the modification of cell membrane permeability causing the leakage of cell contents (Gradel *et al*., 2005; Fazlara and Ekhtelat, 2012). Recently, researchers have studied the effects of different benzalkonium chloride compounds on hatching eggs (Aygun *et al*., 2012a,b). This intervention practice is still much less frequently used in the US than formaldehyde fumigation. Bac-D is a novel disinfectant, which is currently being used as a wound wash for humans and animals and the active ingredient is benzalkonium chloride. Bac-D is a safe, potential substitute to harsh chemicals and it may be capable of decreasing bacteria and improving hatchability. Much investigation is needed in order to determine the specific biocidal effects of Bac-D on different bacteria, viruses and protozoa, so that a kill spectrum may be established.

Therefore, the objectives of this trial were to determine the effects of Bac-D on total aerobic bacterial counts and to explore the claim that Bac-D has an effective killing time of over 3 h.

MATERIALS AND METHODS

Experimental design: Broiler breeder eggs were obtained from the North Carolina State University poultry education unit (Raleigh, NC). The eggs were stored for 4 days in an egg cooler at 60 F and 70% relative humidity. The experiment was designed as a 3×2×2 factorial. There were 3 sampling time points (0, 1.5, 3 h), 2 spray treatments (Bac-D and water) and inoculum (inoculated vs. uninoculated). This design resulted in 12 treatments consisting of 50 eggs each for a total of 600 eggs. The 4 treatment groups for each time point were: Bac-D, inoculated; Bac-D, uninoculated; DI water, inoculated; and DI, uninoculated.

Inoculum: Fresh inoculum was prepared on the day of sampling by placing 25 eggs from the same broiler breeder flock in a sterile container with 40 mL of PBS (Fisher Scientific, Waltham, MA). The eggs were shaken for 60 s and then removed and the inoculum was placed in a sterile 1 L bottle at room temperature until inoculation.

Sampling: Eggs were placed in a sterile plastic egg flat. Each flat was then sprayed with approximately 60 mL of either sterile DI water, or Bac-D. The eggs were incubated at room temperature for 0, 1.5, or 3 h. At the end of their corresponding time points, 50 eggs were inoculated with 40 µL of the endogenous inoculum (2.98 log CFU/mL), which was evenly spread over the surface of the egg with a sterile cotton swab. The inoculum was allowed to air dry for 10 minutes and then the egg was placed in a 710 mL Whirl-pak bag (Nasco, Fort Atkinson, WI) that contained 20 mL PBS (Fisher Scientific, Waltham, MA). The bags were manually shaken for 60 s, then the egg was removed, 40 µL of rinsate were plated on TSA (Becton Dickinson, Sparks, MD) and spread with disposable L-spreaders (Fisher Scientific, Waltham, MA). Plates were incubated at 35 C for 24 h. At the end of the incubation period, colonies were enumerated and recorded and log CFU/mL were calculated.

Conductance and hatchability: A group of 50 eggs from each treatment were labeled and the eggs were weighed on day 0 prior to incubation and again on day 7 of incubation. These weights were used to calculate the relative moisture loss, as well as egg shell conductance. The average conditions for the 7 days were temperature of 99.5F, barometric pressure of 30.11 and relative humidity of 50%. The conductance was calculated using the equation of Paganelli *et al.* (1974). Hatchability was calculated at hatch, the un-hatched eggs were broken out to determine if the eggs were infertile or time at which they had died.

Statistical analysis: The experimental data were analyzed as a 3×2×2 design. The bacterial counts obtained as log CFU/mL based on the variables: sampling times (0, 1.5, 3 h), spray (Bac-D, water) and inoculum (inoculated vs. uninoculated) were analyzed by ANOVA (JMP 10, SAS, Cary, NC) using Tukey-Kramer comparison of means. Broiler breeder eggs were the experimental unit and an alpha of 0.05 was used to establish significance.

RESULTS

The effect of Bac-D as a disinfectant is evident when comparing the mean log CFU/mL of Bac-D washed eggs to eggs washed with DI water only. There was a significant decrease in total aerobic bacteria ($p<0.0001$). Bac-D washed eggs yielded a mean log CFU/mL of 2.08 and water washed eggs, a mean of 3.64 log CFU/mL. This was approximately a 1.5 log CFU/mL reduction in total aerobic bacterial counts (Fig. 1).

Inoculated eggs treated with Bac-D had significantly lower mean log CFU/mL counts at the $p<0.0001$ level for all 3 time points of the inoculated group when compared to the control. At the different sampling times (0, 1.5, 3 h) the bacterial counts were 1.96, 2.60, 1.37 log CFU/mL, respectively for Bac-D treated eggs. The total aerobic bacteria counts for control eggs at the same sampling times were 3.76, 3.66, 3.55 log CFU/mL (Table 1).

The same pattern was observed in uninoculated eggs. Bac-D treated eggs had significantly lower bacterial counts ($p<0.0001$) at 1.5 and 3 h (2.36, 2.14 log CFU/mL). Eggs washed with DI water had significantly higher ($p<0.0001$) bacterial counts when compared to Bac-D washed eggs: 1.5 h, 3.62; 3 h, 3.63 log CFU/mL (Table 2).

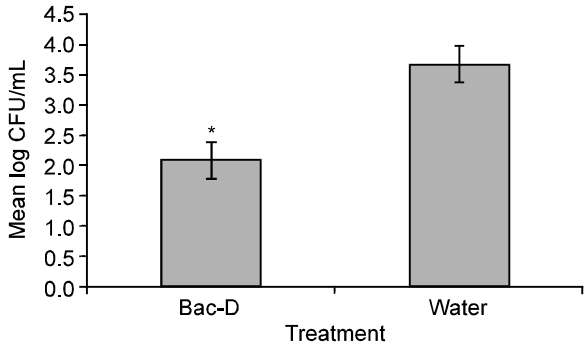

Fig. 1: Overall Comparison of Mean log CFU/mL by Treatment (Bac-D vs., Water) of Inoculated and Uninoculated Eggs at all Sampling Time Points (0, 1.5, 3 h).
*Means differ significantly (p<0.0001)

Table 1: Mean log CFU/mL comparison of total aerobic bacteria on inoculated eggs sprayed with bac-d vs. Water at different sampling times

	0 h		1.5 h		3 h	
Sampling times	Mean	S.E	Mean	S.E	Mean	S.E
Bac-D	1.96^{B}	0.131	2.60^{B}	0.134	1.37^{B}	0.133
Water	3.76^{A}	0.142	3.66^{A}	0.134	3.55^{A}	0.136

^{A,B}Means within columns with different superscripts differ significantly (p<0.0001)

Table 2: Mean log CFU/mL comparison of total aerobic bacteria on uninoculated eggs sprayed with bac-d vs. Water at different sampling times

	1.5 h		3 h	
Sampling times	Mean	S.E	Mean	S.E
Bac-D	2.36^{B}	0.137	2.14^{B}	0.136
Water	3.62^{A}	0.146	3.63^{A}	0.141

^{A,B}Means within columns with different superscripts differ significantly (p<0.0001)

Table 3: Mean log CFU/mL comparison of total aerobic bacteria on Inoculated and uninoculated eggs sprayed with Bac-D vs. Water at different sampling times

Sampling times	0 h		1.5 h		3 h	
Treatment	Bac-D	Water	Bac-D	Water	Bac-D	Water
Mean CFU	1.96^{C}	3.78^{A}	2.48^{B}	3.65^{A}	1.75^{C}	3.59^{A}
SE	0.133	0.144	0.097	0.100	0.096	0.099

^{A,B,C}Means with different superscripts differ significantly (p<0.0001)

When samples from both treatments were pooled, Bac-D washed eggs had significantly lower total aerobic bacterial counts at all 3 sampling time points: 0, 1.5 and 3 h with an average of 1.96, 2.48 and 1.75 log CFU/mL. On the other hand, eggs washed with DI water at the same sampling points produced 3.78, 3.65 and 3.59 log CFU/mL, respectively (Table 3).

There were no significant differences in hatchability or hatch residue counts, however there was a significant (p<0.0468) change in conductance of the eggs. The eggs that were washed with Bac-D had a slightly higher conductance rate than did the water washed eggs (Table 4).

Table 4: Hatchability, Conductance and hatch residue breakout from eggs washed in Bac-D or water

	Infertile	Early Dead	Mid Dead	Late Dead	H	C
Bac-D	16	4	2	13	76.6%	0.0156^{A}
Water	24	2	2	11	74.0%	0.0147^{B}

^{A,B}Means within columns with different superscripts differ significantly (p<0.0001). H: Hatchability, C: Conductance

DISCUSSION

Researchers have found benzalkonium chloride to be an effective disinfectant when used as a wound wash for animals and humans (Dyer *et al*., 1998). This may be due to its biocidal properties on different organisms found in the environment (Houari and Martine, 2007). Overall, when results for eggs both inoculated and uninoculated were pooled, averaged and compared only between treatments (Bac-D vs. water), there was approximately a 1.5 log CFU/mL reduction of total aerobic bacteria on the exterior of eggs when Bac-D was used. This reduction was lower than a study performed by Romanova *et al*. (2007) who demonstrated a reduction of approximately 4 log CFU/mL when benzalkonium chloride was applied for 30 minutes at a rate of 1 mg/mL on *Listeria monocytogenes* biofilms. This may be due to increased sensitivity of *L. monocytogenes* to benzalkonium chloride, as well as, the different composition of the disinfectant tested. Similar results were found by Velazquez and coworkers (2009) who showed significant reductions of *Yersinia enterocolitica* and *Escherichia coli* O157:H7 in tomatoes and lettuce treated with a solution containing 0.1 mg/mL of benzalkonium chloride. Aygun and Sert (2012) tested the efficacy of benzalkonium chloride on hatching eggs and determined that total aerobic mesophilic bacteria were significantly reduced compared to the control treatment (water) after 7 and 14 d of treatment. These results coincide with our findings; longer exposure to treatment yielded lower total aerobic bacteria counts. It may be worth noting that this investigation only compared 3 sampling time points (0, 1.5 and 3 h). Future work should focus on extended treatment times, which may result in greater total aerobic bacteria log CFU/mL reductions. Kuda *et al*. (2007) established that the presence of organic materials such as milk and beef and tuna gravy inhibited the efficacy of benzalkonium chloride on biofilms of *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is in agreement with results found by Klimek and Bailey (1955) and may be the reason why our work did not yield higher log CFU/mL reductions in unwashed eggs. Kuda *et al*. (2007) determined that using benzalkonium chloride after a wash resulted in non-detectable bacterial counts.

Therefore, a wash and disinfectant process should be investigated in order to achieve lower bacterial loads in hatching eggs.

Bac-D is an intervention method worth exploring by the egg industry since it does not have the carcinogenic concerns of formaldehyde and other inconveniences of fumigation (Adler *et al*., 1979). Bac-D is a new product with disinfectant properties, which warrants more investigation. Future work must focus on testing different product concentrations, as well as, impact on hatchability. Furthermore, the development of Bac-D as a disinfectant for other poultry-related uses, such as house cleanouts is paramount in order to mitigate the need for alternative sanitizing solutions.

ACKNOWLEDGEMENTS

The authors would like to thank RDM Products for providing the Bac-D used for this trial. We are also greatly indebted to the Charles Lee Guy fund for financial support.

REFERENCES

Adler, H.E., A.J. DaMassa and W.F. Scott, 1979. Studies on egg disinfection. Poult. Sci., 58: 799-806.

Aygun, A. and D. Sert, 2012a. Effects of ultrasonic treatment in eggshell microbial activity, hatchability, tibia mineral content and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. Poult. Sci., 91: 732-738.

Aygun, A. and D. Sert, 2012b. Effects of propolis on eggshell microbial activity, hatchability and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. Poult. Sci., 91: 1018-1025.

Brake, J. and B.W. Sheldon, 1989. Effects of A Quaternary Ammonium Sanitizer for Hatching Eggs on Their Contamination, Permeability, Water Loss and Hatchability. Poult. Sci., 69: 517-525.

Coufal, C.D., C. Chavez, K.D. Knape and J.B. Carey, 2003. Evaluation of a Method of Ultraviolet Light Sanitation of Broiler Hatching Eggs. Poult. Sci., 82: 754-759.

Dyer, D.L., K.B. Gerenraich and P.S. Wadhams, 1998. Testing a new alcohol-free hand sanitizer to combat infection. AORN J., 68: 239-251.

Fasenko, G.M., E.E. O'Dea Christopher and L.M. McMullen, 2009. Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters. Poult. Sci., 88: 1121-1127.

Fazlara, A. and M. Ekhtelat, 2012. The disinfectant effects of benzalkonium chloride on some important foodborne pathogens. American-Eurasian J. Agric. and Environ. Sci., 12: 23-29.

Funk, E.M., J. Forward and M. Lorah, 1954. Minimizing Spoilage in Shell Eggs by Thermo-Stabilization. Poult, Sci., 33: 532-538.

Gradel, K.O., L. Randall, A.R. Sayers and R.H. Davies, 2005. Possible associations between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a putative role of mar. Vet. Microbiol., 107: 127-138.

Gerald, L.B., J.K. Gerald, L.A. McClure, K. Harrington, S. Erwin, W.C. Bailey, 2011. Redesigning a large school based clinical trial in response to changes in community practice. Clin. Trials, 8: 311-319.

Houari, A. and P. Di Martino, 2007. Effect of Chlorhexidine and Benzalkonium Chloride on Bacterial Biofilm Formation. Lett. Appl. Microbiol., 45: 652-656.

Klimek, J.W. and J.H. Bailey, 1955. Factors influencing the rate of killing of Escherichia coli exposed to benzalkonium chloride. Appl. Microbiol., 4: 53-59.

Kuda, T., T. Yano and M.T. Kuda, 2007. Resistances to benzalkonium chloride of bacteria dried with food elements on stainless steel surface. LWT, 4: 988-993.

Lorenz, F.W., P.B. Starr and R. Bouthilet, 1950. Fumigation of Shell Eggs with Ethylene Oxide. Poult. Sci., 29: 545-547.

Miller, W.A., 1956. The Effect of Coating the Shells of Washed Eggs that Formerly were Dirty,with Antibiotics, Upon Subsequent Spoilage. Poult. Sci., 35: 241-243.

Peebles, E.D. and J. Brake, 1985. The Role of the Cuticle in Water Vapor Conductance by the Eggshell of Broiler Breeders. Poult. Sci., 65: 1034-1039.

Paganelli, C.V., A. Olszowka and A. Ar, 1974. The avian egg: Surface area, volume and density. Condor, 76: 319-325.

Romanova, N.A., Gawande, P.V., Brovko, L.Y. and M.W. Griffiths, 2007. Rapid methods to assess sanitizing efficacy of benzalkonium chloride to Listeria monocytogenes biofilms. J. Microbiol. Methods, 71: 231-237.

Sheldon, B.W. and J. Brake, 1990. Hydrogen Peroxide as an Alternative Hatching Egg Disinfectant. Poult Sci., 70: 1092-1098.

Velazquez, L.C., N.B. Barbini, M.E. Escudero, M.L. Estrada and A.M. Stefanini, 2009. Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing Escherichia coli O157:H7 and Yersinia enterocolitica on fresh vegetables. Food Control, 20: 262-268.

Whistler, P.E. and B.W. Sheldon, 1988. Bactericidal Activity, Eggshell Conductance and Hatchability Effects of Ozone Versus Formaldehyde Disinfection. Poult. Sci., 68: 1074-1077.