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Sanitation of hatchings eggs in hatcheries by using Decon7 as a fogging agent

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Introduction:

Water supply in poultry hatcheries need to be sanitized as contaminated water can cause severe bacterial or fungal problems. The slow moving warm water in hatcheries is a perfect environment for different microbial pathogens to proliferate. Contaminated water coming in contact with hatching eggs or newly hatched chicks by way of humidification or environmental fogging lead to infections in chicks either through inhalation or ingestion. This can result in either a significant loss of hatchability or an increase in first week mortality. Poultry industry makes use of disinfectants to eliminate / reduce the microbial population. Formaldehyde the age old disinfectant used in hatcheries due to its cost effectiveness is effective against the microbes but the health risks associated with this chemical are numerous. Another inexpensive disinfectant used in poultry industry to decontaminate water is chlorine, which is readily available in an inexpensive form, the liquid bleach (sodium hypochlorite). The limiting factor in the use of chlorine is that it works best as a sanitizer in an acidic environment which can be detrimental to shell integrity. Decon7, a complex mixture of organic and inorganic chemicals is a decontamination product created by the Sandia National Laboratories, and approved for use in hatcheries and food processing facilities. The product has three parts: Part 1, a combination of 3rd and 4th generation quaternary ammonia at 3 % concentration; Part 2, 7.9% hydrogen peroxide and Part 3, diacetin. Depending upon the application, part 1 and 2 are either mixed alone at equal rate, or they are mixed with part 3 at 2% rate. Decon7 has been found to be effective against a wide range of microbial contaminants with a contact time of ten minutes. The advantages of this product include sporicidal action, non-corrosiveness, biodegradability and a longer residual activity. The objective of our project is to evaluate Decon7 as a sanitizer and to determine the concentration of the product required to effectively control microbial contamination introduced through the water supply or eggs within hatcher cabinets.

Materials and Methods:

Trial 1 - Laboratory study: Ninety non-fertile eggs were obtained from leghorn type strain of hen flock located on the UA Poultry Research Farm. These eggs were stored at 68ºF post lay to simulate hatching egg storage conditions, after which they were sprayed with a slurry of water and chicken feces, (100 part water 10 parts chicken feces), allowed to dry and then placed randomly into 9 incubators at the rate of 30 eggs per incubator and warmed to incubation temperature (99.5ºF**). The slurry spray was serially diluted and plated for aerobic bacterial count, *E.coli*, yeast and mold.** The incubators were randomly assigned to one of the three following treatments with 3 incubators within a treatment group: (1) control; (2) Formaldehyde and (3) Decon7 (1 oz each of parts 1and 2 mixed with 1.2 ml of part 3). Ten eggs were sampled pretreatment, marked as sampled and the remaining 20 eggs/treatment were sampled 8 h and 24 h post final treatments with 10 eggs per sampling time. Egg sampling was done by aseptically removing the egg from the incubator and placing it into 30 ml of sterile BPD in a whirl-pack bag where it was gently rinsed and agitated in the BPD for 15 seconds. The egg were aseptically removed from the BDP rinse and the BPD rinse was then cultured for most probable aerobic plate count, *E. coli*, yeast and mold.

Trial 2 - The University of Arkansas Poultry Research Farm study: 9 Hova Bator incubators designed to hold approximately 50 eggs and equipped with circulation fans, heaters and thermostats were used for this study. 450 nest eggs were obtained from a broiler breeder farm, transported to the U of A facility and stored at 67ºF until set**.** The eggs were randomly assigned to one of the three treatments (control, formaldehyde and Decon7), marked with the treatment number and then placed onto setter trays so that all treatments are equally distributed on the racks. The eggs were then incubated for 17 days after which the eggs were pulled out for candling to assure fertile embryos and then placed by treatment in 9 incubators (3 incubators/150 eggs/treatment). The incubators were swabbed prior to use to determine most probable count of aerobic bacteria, *E. coli*, yeast and mold. 10 eggs were randomly selected on day 18 for pretreatment sampling as in Trial 1, after which they were dried and placed into the appropriate

incubators where they received the respective treatments. Each egg was rinsed in BPD similar to the previous procedure and returned to the incubators for the remainder of the incubation process. The BPD rinse of the pretreatment eggs was cultured for most probable aerobic plate count, *E. coli*, yeast and mold to estimate the initial counts. The treatments were started on day 18 after pre-treatment sampling and the last treatment was done at 7 pm on day 19 as one bird had hatched out at that time. Eggs were sampled 4 h after the last treatment on day 19. The newly hatched out birds were placed in pens on days 20 and 21 after they were 80% dry. After the placement of chicks in pens the lids of the incubators were aseptically removed and the inside of the lids swabbed for microbial analysis. Later on break outs of eggs that had not hatched were done to analyze the reasons for no hatch. The incubators with hatched egg shells were again sprayed with the respective treatments. Four hours post-treatment the lids of the incubators were swabbed and swabs were analyzed for most probable aerobic plate count, *E. coli*, yeast and mold.

Application rate of sanitizers

1. **Control- 19.8 ml of deionized water was placed in an open container (weigh boat) each time the other two treatments were applied. No disinfectants were added to the incubator prior to the eggs hatching.**
2. **Formaldehyde- A total of three doses were given at 12 hr intervals. Each dose consisted of 3 mL of 37% formaldehyde added to 19.8 ml of deionized water and placed in an open container (weigh boat). The mixture was allowed to evaporate inside the incubators**
3. **Decon7 spray application- 1 oz each of parts 1 and 2 were mixed with 1.2 ml of part 3 and this mix was then fogged as a very fine spray into the incubator for three treatments 4 hours apart after the eggs were placed in the incubators. A total of 9 treatments were applied to the incubator before ceasing at the sign of the first egg pipping.**

Application of sanitizers was stopped immediately after the hatching of the first egg late on day 19. Temperature and humidity was constantly monitored throughout the hatch process. The temperature goal was the range of 99-101°F and humidity target was 50-55% in all treatments.

Laboratory analysis

The BPD rinses of eggs in trial 1 and 2 were cultured for aerobic bacteria, *E. coli*, yeast and molds using serial dilution and plating onto 3M Petrifilm™. Microbial counts were enumerated after appropriate times of incubation (48 h at 35° C for APC; 48 h at 37° C for *E. coli* and 72 h at 20° C for yeast and mold).

Hatchability and performance

The hatched chicks were removed, counted to determine percent hatch and then group placed (by incubator) into floor pens where birds received a common starter diet. All the un-hatched eggs were broken out to investigate the possible reason and time of death. Two chicks per treatment were sacrificed at day of age, tracheal tissue collected and placed into formalin. Tissue samples have been sent to OSU for histology analysis. All the chicks were group weighed by pens at days 0, 7 and 14. Chicks received a starter feed that was formulated to meet the nutritional requirements of the Cobb 500 broiler. All feed added was weighed and feed remaining in the pens was weighed on days 7 and 14. All chicks which died or were culled were weighed and this weight was used for calculation of an adjusted feed conversion for days 0-17, 7-14 and 0-14 days.

Results

For trial 1 which utilized the non-fertile eggs that had been lightly sprayed with the fecal slurry prior to placement into the incubators, a significant reduction (P=.0001) in the APC counts at 8 h and 24 h post last treatment were observed for both formaldehyde and Decon7 as compared to the Control or untreated eggs (Table 1, Figure 1). By 8 hours post last treatment there was a three log reduction in APC levels and counts were undetectable for both treatments at 24 hours while

almost 1 log was still detectable for the control. Levels of yeast, mold and E coli were negligible for initial counts and went down to un-detectable levels at 8 and 24 hours post treatment (Table 2).

For trial 2, the initial microbial counts on the shells of the fertile eggs was much lower than the egg microbial loads utilized in trial 1 and were very similar for all treatments, ranging from 1.2 to 1.35 log10 for APC. However, post incubation shell rinses saw a 2.4 log increase in the control egg shell apc microbial levels and formaldehyde increased apc levels to 2.088 log 10 while the Decon 7 apc levels dropped to .961 log 10 (Table 3). Levels of yeast, mold, E. coli and coliforms were minimal initially (~.2-.38 log 10) but both formaldehyde and Decon 7 both lowered mold whereas the control experienced increased surface shell mold levels. (Table 3). The counts for aerobic bacteria in the incubators 4 h post last Decon 7 treatment were not statistically lower than the formaldehyde or control treatments but it was numerically lower and the difference was approaching statistical difference with a p Value of .1564. And this trend continued in the incubator 4 hours after the hatch was pulled. No differences were seen in the incubators for yeast and mold levels. (Table 4). When an analysis of treatment, regardless of time, impact on incubator microbial recovery was done, it showed that both Decon 7 and Formaldehyde both had significantly lower APC levels than the control incubators (Table 5, P=.0022). No treatment differences were observed for mold or yeast.

The hatchability for the Decon7 treatment groups was lower but not significantly different from the other treatment groups (Table 5). A breakout of the un-hatched eggs showed that Decon 7 did have a numerically higher number of rinsed eggs (eggs sampled for pre and post shell microbial levels) which did not hatch as compared to the other treatments and that late dead for the Decon7 was 11.33 % (17 eggs out of the 150 set) as compared to the control which had .667% (1/150 eggs) and formaldehyde with 3.33% (5/150 eggs) late embryo dead. It is possible that the treatment, combined with the rinsing of the eggs in the BPD could have created an air exchange barrier which impacted the late stage embryos (Table 6). No differences were observed for 0, 7 or 14 day weights, feed conversion or 0-7 or 0-14 day liveability. However we did not find any significant differences in average body weight, average weight gain, average

feed: gain ratio, average feed intake per bird and livability. No chicks died in the Decon7 treatment for the 0-14 day period. (Tables 7 – 11) Tracheal histology is not completed yet.

Conclusion

Microbial counts for the non-fertile eggs used in Trial 1 were non-detectable 24 h post treatment with Decon7 whereas in Trial 2 the aerobic plate counts were found to be 2.7 logs lower than the control group and 1.1 logs lower than the formaldehyde treated groups. Swab evaluations of the incubators pre-treatment, post-hatch and 4 h post-treatment did show Decon7 as effective as formaldehyde in reducing the APC load. While late dead embryo mortality was higher with the Decon 7, chick livability was excellent for this treatment so the late dead embryo mortality is most likely related to the treatment, egg rinse. A second study without egg rinsing should be conducted to confirm this. Use of the formaldehyde or Decon7 did not impact the bird performance in any way.

Table 1: Trial 1-Impact of hatcher treatments on the egg shell surface APC and yeast levels on the shell surface of non-fertile eggs-Initial evaluation

Incubator Treatment	Microbial Levels (log_{10})					
	APC[1]			Yeast		
	Pre-Treatment	8 Hours Post Treatment	24 Hours Post Treatment	Pre-Treatment	8 hours Post Treatment	24 Hours Post Treatment
Control	2.976_a	1.41_b	$.948_c$	1.01	.208	0.0
Formaldehyde	2.917_a	$\mathbf{.030_d}$	0.00_d	.94	0.0	0.0
Decon7	3.047_a	$\mathbf{.030_d}$	0.00_d	1.15	0.03	0.0
SEM	.126			.034		
***P* Value**	.0001			0.0932		

1. Values with different letters in columns for APC levels are significantly different at the P<0.05 level

Table 2. Trial 1-Impact of hatcher treatments on the egg shell surface mold and E coli levels for non-fertile eggs-Initial evaluation

Incubator Treatment	Microbial Levels (log_{10})					
	Mold			E coli		
	Pre-Treatment	8 Hours Post Treatment	24 Hours Post Treatment	Pre-Treatment	8 hours Post Treatment	24 Hours Post Treatment
Control	.38	.12	0.0	.16	0.0	0.0
Formaldehyde	.39	.03	0.0	0.0	0.0	0.0
Decon7	.41	.095	0.0	.25	0.0	0.0
SEM	.072			.05		
***P* Value**	.9618			0.0803		

Figure 1: Aerobic bacterial counts pre and post-treatment for non-fertile eggs

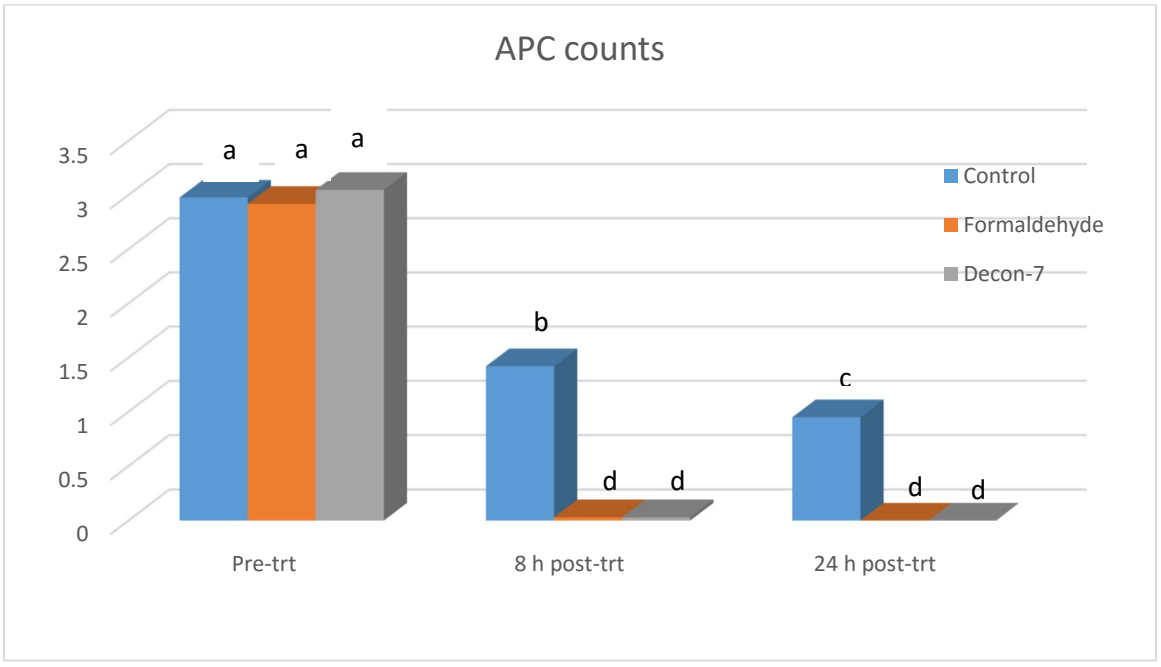

Table 3: Trial 2-Impact of treatments on egg shell surface microbial levels pre- and post-treatment for fertile eggs treated during last stage of incubation

Treatment	Shell Surface Microbial Levels (log_{10})									
	APC		Yeast		Mold		E. coli		Coliforms	
	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt
Control	1.20_c	3.676_a	0.032	0.134	0.152_b	$.324_a$	0.000	0.000	0.026	0.026
Formaldehyde	1.30_c	2.088_b	0.016	0.000	0.072_c	0.020_c	0.000	0.000	0.000	0.000
Decon7	1.35_c	0.961_c	0.000	0.000	0.086_c	0.026_c	0.000	0.000	0.000	0.000
SEM	.148		.037		.039		0		.0150	
***P* Value**	**0.0001**		**0.1155**		**0.0040**		**1.000**		**1.000**	

1. Values within each microbial species which have different letters are statistically different at the P<0.05 level

Table 4: Trial 2-Impact of treatments on recoverable microbial levels from swabs of the incubators post hatch and 4 hours post the last Decon 7 Treatment

Treatment	Microbial Levels (log_{10})								
	APC			**Yeast**			**Mold**		
	Pre Trt	**4 h Post last Trt**	**4 h Post Hatch**	**Pre Trt**	**4 h Post Last Trt**	**4 h Post Hatch**	**Pre Trt**	**4 h Post Last Trt**	**4 h Post Hatch**
Control	1.194	1.968	2.404	0.000	0.1000	0.000	0.382	0.434	0.993
Formaldehyde	1.232	0.634	1.018	0.000	0.333	0.000	0.233	1.031	0.945
Decon7	1.045	0.159	.301	0.000	0.201	0.000	0.201	0.983	0.959
SEM	.402			.133			.323		
P Value	0.1564			0.9036			0.7343		

Table 5. Trial 2-Impact of treatment on the microbial recovery of swabs on the interior surface of the incubators (pre and post times combined)

Incubator Treatment	APC	Mold	Yeast
	(Log_{10})		
Control	1.855_a	.603	.033
Formaldehyde	0.96_b	.736	.11
Decon7	0.50_b	.714	.067
SEM	.23	.186	.077
P Value	.0022	.8640	.7786

Table 6: Trial 2-Impact of hatcher treatment on hatchability (%), Embryo Mortality at different stages of development (%) and cull chicks (%)

Incubator Treatment	Hatchability	Un-hatched eggs rinsed for microbial	Early Dead	Mid Dead	Late Dead	Chicks Pipped but not hatched	Cull Chicks
	% of eggs placed (Actual number /150 eggs)						
Control	94.667 (142/150)	1.07 (1/60)	0.667 (1/150)	0.000	0.667 (1/150)	2.667 (4/150)	1.333 (2/150)
Formaldehyde	91.333 (137/150)	5.0 (3/60)	1.333 (2/150)	0.000	3.333 5/150)	2.667 (4/150)	1.0 (2/150)
Decon7	86.000 (129/150)	10.0 (6/60)	0.667 (1/150)	0.000	11.333 (17/150)	2.000 (3/150)	0.0 (0/150)
SEM	2.15	3.85	.94	0	2.90	1.44	.86
p-value	**0.194**	**.3677**	**0.8503**	**1.0000**	**0.0918**	**0.9318**	**0.4921**

Table 7: Trial 2-Impact of hatcher treatment on body weights of broiler chicks

Treatment	Day 0	Day 7	Day 14
	Average Weight (g/bird)		
Control	46	151	378
Formaldehyde	46	151	376
Decon7	45	153	381
SEM	.03	3	8
p-value	0.4524	0.9249	0.8874

Table 8: Trial 2-Impact of hatcher treatment on average broiler chick weight gain

Treatment	Day 0-7	Day 7-14	Day 0-14
	Average Weight Gain (g/bird)		
Control	105	226	332
Formaldehyde	105	224	330
Decon7	108	228	336
SEM	3	6	8
p-value	0.8832	0.9147	0.8690

Table 9: Trial 2-Impact of hatcher treatment on average adjusted feed:gain ratios (g:g) of broiler chicks

Incubator Treatment	Day 0-7	Day 7-14	Day 0-14
	Feed:Gain Ratio (g:g)		
Control	1.222	1.301	1.276
Formaldehyde	1.238	1.293	1.292
Decon7	1.200	1.309	1.275
	.02	.011	.01
p-value	0.5476	0.6249	0.4895

Table 10: Trial2-Impact of hatcher treatment on average feed intake (kg/bird) of broiler chicks

Incubator Treatment	Day 0-7	Day 7-14	Day 0-14
	Intake (g/bird)		
Control	128	295	423
Formaldehyde	135	290	426
Decon7	129	299	428
SEM	4	7	8
p-value	0.5133	0.7364	0.9267

Table 11: Trial2-Impact of hatcher treatment on chick livability

Treatment	Day 0-7	Day 0-14
	(% Livability or Remaining)	
Control	99.31	99.31
Formaldehyde	100.00	99.22
Decon7	100.00	100.00
SEM	.40	.60
p-value	0.4219	0.6281