Preliminary Egg Sanitization Studies

March 2014

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Introduction

Turkey eggs are collected in the laying barns from nests and other spots where they are placed by the hens. The collected eggs are held in holding rooms at about 50% humidity and temperatures between 50 and 75ºF for periods of up to two weeks. During this time microbes on the surface of the eggs may multiply. After holding the eggs are moved into incubators at higher temperature and higher humidity, which is expected to accelerate the growth of microbes on the outer surface of the eggs. Upon hatching the poults are in contact with the exterior of the shells and may become infected by the bacteria on the shells. This infection may decrease the early survival of the poults.

Project Plan

HGI has a technology which produces an airborn stream of reactive chemical compounds that have been found to decrease the airborn infectious agents. This technology is also effective in decreasing the concentration of odiferous compounds. Consequently, the primary applications of the HGI technology have been in odor mitigation where it has been highly effective. Exposure of turkey eggs to the stream of reactive chemical compounds is proposed to be an effective sanitizing strategy. Decreasing the number of infectious microbes on the outer surface of the turkey eggs is expected to increase the survival rate of young poults.

The HGI technology uses an intense UV light source to convert water molecules from humid air (>40% RH) into hydroxyl radicals which will react with many compounds in the gas phaseand on surfaces.
Reactions with the primary compounds in the atmosphere will produce compounds which will be nearly as reactive as hydroxyl radicals and a chain of chemical reactions is proposed to produce a variety of reactive compounds capable of disrupting microbial growth. These compounds are also reactive with most odiferous compounds which is the mechanism for the demonstrated odor mitigation of the HGI technology.

Design of Studies

The initial study included two small hydroxyl radical generators (HRGs) in a small room (12' x 16') for the test condition. A room humidifier and thermostated building HVAC system were used for temperature and humidity control. 150 unwashed turkey eggs in tray of 30 eggs were placed on a table in the center of the room with HRGs placed on either end of the table. The control eggs (150 total) were placed on a table in a larger room, but under a tent to allow control of the humidity. Eggs were sampled from test and control conditions on the following schedule;

	Control		**Test**
Day 0	30 eggs	0	At Day 0 the Test and Control eggs are identical
Day 1	30	30	
Day 2	30	30	
Day 3	30	30	
Day 9	30	30	

Sampled eggs were tested in sets of ten eggs as follows. Individual eggs were washed in 5 ml of dilute phosphate buffer. 1 ml of the washing buffer was combined with 1 ml of washing buffer from each of the other 9 eggs in the set to produce a composite washing solution of 10 ml. This composite solution was diluted (1:1000, 1:10000, 1:100000) and 1 ml of each dilution was plated on Petrifilm plates. The plates were incubated for three days and the number of colonies per plate was counted. These results reflect the number of viable microbes per ml of diluted washing buffer. All dilutions were with dilute phosphate buffer (0.01 M, pH = 7.5).

At some time points, the composite washing buffer (10 ml per set of 10 eggs) from the three sets of ten eggs was pooled (3 x 2ml per set = 6 ml) with other washing buffer composites from the other two sets of eggs to minimize the number of plates and decrease the time requirements for counting and averaging the colonies.

A subsequent study was designed using washed eggs. The configuration of the test condition was also revised for the washed egg study. The test condition in the same room was modified to include a tent over the table with the HGRs and the humidifier inside of the tent. Higher humidity was generated and the exposure to the HGR air stream was increased. Additionally, the egg trays were elevated 2" above the table on open stands to allow greater airflow around the eggs.

Sampling of the eggs from the washed egg study was as follows;

	Control	Test
Day 0	30 eggs	0
Day 1	30	30
Day 2	30	30
Day 3	30	30
Day 18	30	30

The washing from this subsequent study were diluted (1:1, 1:100, 1:1000) and 1 ml of each solution (undiluted and three dilutions) were plated on Petrifilms . After a minimum of two days of incubation, the plates were counted.

Results and Discussion

The study of unwashed eggs produced results (Table 1) that did not support the expectation of improved sanitization of turkey eggs by exposure to the HRG. Temperature in both conditions was controlled between 68ºF and 72ºF. The humidity in the Control tent was constant at 45%. The humidity in the Test condition room was about 25% and the humidifier was overwhelmed by the building air system. In both conditions, there was a modest decrease in the viable microbes on the surface of the eggs. Counts of the individual sets of ten eggs (vs. a composite of the washing solutions) showed acceptable reproducibility and minimal variability between sets of eggs. It was observed that there was considerable material on the eggs and the washing solutions might be described as muddy.

	Composite Liquid Washings								
	Day 0	Day 1		Day 2		Day 3		Day 9	
		Control	Test	Control	Test	Control	Test	Control	Test
1000	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10000	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
100000	TNTC	TNTC	TNTC	29	54	41	60	82	75
1000000	250	123	111	12	7	16	19	12	6

Day 3 Individual Sets						
	Set 1		Set 2		Set 3	
	Control	Test	Control	Test	Control	Test
1000	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10000	131	TNTC	114	TNTC	TNTC	TNTC
100000	28	69	12	39	73	50
1000000	1	19	4	10	29	13

Control Average = 38 at 100,000

Test Average = 53 at 100,000

TNTC = Too Numerous To Count
Sterility Control Plate had no colonies.

Table 1 Results with Unwashed Eggs

The study of washed eggs provided results with a clearer indication of a favorable effect from the HRG exposure (Table 2). The humidity in both conditions was held at 45 to 55% and the temperature was controlled between 68º and 72ºF. With the washed eggs the initial washing buffer could be plated to generate a countable result. This indicates that a reduction of viable microbes of about 10000 fold was accomplished by the washing. Eggs in the Control condition showed a modest increase in the number of viable microbes during the course of the study. In contrast, the eggs in the Test condition (HRG exposure) show a measureable decrease in the number of viable microbes. The on individual sets of 10 eggs showed acceptable reproducibility and for

Day 2 and Day 3, the washing buffers were pooled to provide one set of dilution for plating and counting.

	Composite or Average of Three Sets								
	Day 0	Day 1		Day 2		Day 5		Day 18	
Dilution		Control	Test	Control	Test	Control	Test	Control	Test
1	112	137	104	147	91	252	73	TNTC	58
10	24	18	21	32	26	37	16	92	22
100	10	13	16	14	12	18	21	34	26
1000	13	NT	NT	NT	NT	NT	NT	NT	NT

	Individual Sets								
		Set 1		Set 2		Set 3		Average	
	Dilution/Day	Control	Test	Control	Test	Control	Test	Control	Test
	Day 0								
	1	104	NT	124	NT	107	NT	112	NT
	10	28	NT	19	NT	25	NT	24	NT
	100	15	NT	7	NT	9	NT	10	NT
	1000	20	NT	6	NT	12	NT	13	NT
	Day 1								
	1	153	115	123	84	136	112	137	104
	10	24	27	16	13	15	21	18	21
	100	17	21	12	12	9	16	13	16
	Day 18								
	1	TNTC	26	TNTC	79	290	49	TNTC	58
	10	155	17	76	29	47	21	92	22
	100	41	20	24	28	38	31	34	26

TNTC = Too Numerous To Count
NT - Not Tested
Sterility Control Plate had no colonies.

Table 2. Washed Eggs

Conclusion

In these preliminary results, the number of viable microbes on washed eggs increased during the 18 days of storage at room temperature and elevated humidity. This increase was not seen on the eggs exposed to the Hydroxyl Radical Generator (HRG). The results show a decrease in viable microbes on the surface of eggs exposed to the reactive compounds produced by the HRG.

Unwashed eggs did not produce equivalent results. There does not appear to be any trend in the number of viable microbes recovered from the unwashed eggs in either the test or control conditions. This may be due to the thicker layer of material on the eggs or the lower humidity in the test condition for the unwashed eggs. The lower humidity during storage (25%) may have prevented effective generation of reactive compounds by the HRGs. Subsequent studies with unwashed eggs stored at 50% humidity will be needed to investigate the impact of HRGs on unwashed eggs.