Auburn University Egg Holding and Incubation Studies 1 and 2

Wallace Berry
Ken Macklin

Objectives
1. Determine efficacy of dry mist sanitizing technology on bacteria and mold numbers on eggs in the egg holding room prior to incubation and at the time of *in-ovo* inoculation and transfer of eggs to the hatching system.

2. Confirm that the sanitizing method did not adversely affect embryonic survival and to examine for effects on chick mortality during the first seven days following hatching.

Number of Eggs
Study 1: 1133
Study 2: 4000 (approx.)

Location and Facilities
AU Poultry Research Unit "Battery House" rooms 10 and 11. Rooms were equipped with AC units and humidifiers to provide a stable temperature of 67°F and 70% relative humidity. Each room was equipped with a Natureform NMC 2000 incubator. Room 10 housed and incubated the control eggs and Room 11 housed and incubated the treated eggs in Study 1. The treatments and rooms were switched for Study 2.

Procedures
Fertile hatching eggs were sourced from commercial hatcheries. Eggs in both studies were from Aviagen 708 strain hens. These are among the most common strain of broiler chickens grown in the US. In both studies, the eggs were distributed into incubator racks and placed in two separate egg storage rooms and held at 65-67°F and 70% relative humidity to simulate storage conditions at commercial hatcheries. Duration of egg storage was two days. Dry mist sanitation commenced at developer's discretion.

Following the storage phase, the eggs were placed in incubators (one in each room) and incubated at 99.5°F and 65% relative humidity until simulated *in-ovo* inoculation at 18 days of incubation. The simulated *in-ovo* inoculation consisted of punching a hole in the large end of each egg with an 18 gauge needle. Eggs were sanitized before and after inoculation at developer discretion. Following *in-ovo* inoculation, the eggs were transferred to hatching trays and placed back into the incubators and allowed to hatch. Dr. Macklin's group took sample eggs from the incubators for microbiological monitoring at several times during incubation.

"Residue" Analysis was conducted on the remaining unhatched eggs to determine the proportion of eggs that were infertile and the number of embryos that died during incubation. Dead embryos were categorized by the time of embryonic death and whether they were contaminated by bacteria or mold.

In each study, four hundred chicks per treatment were placed in 32 broiler pens (bedded with pine shaving typical of commercial broiler housing) and reared for two weeks to determine initial growth rate and chick livability. In Study 1, the chicks were placed in pens containing pine shavings that had already had a flock of chickens grown on them. This was to conform to typical practice in the commercial broiler industry. In Study 2, pens with used bedding were not available so clean pine shavings were used. Chick body weights were recorded at the time the chicks are

pulled from the incubator and again at one week of age. Chick mortality was recorded daily. Feed consumption was measured at 2 weeks of age.

Data Collection

1. Microbiological surveys – Dr. Macklin surveyed microbial populations on eggs before and during storage, before and after *in-ovo* inoculation.
2. Egg weights before and after storage.
3. Mold presence/absence in eggs following *in-ovo* inoculation.
4. Chick hatching rate, embryonic mortality analysis (when embryos died).
5. Chick weight at hatching and end of experiment.
6. Chick feed consumption and feed efficiency
7. Chick mortality.

Results

Trial 1

Hatching Results and Residue Analysis: Hatching and residue analysis results are presented in Table 1. Treated eggs were subjected to the sanitizing treatment. Control eggs received no sanitizing treatment. The Percent Hatched is the percentage eggs that hatched out of the total number of eggs that were incubated. Hatch of Fertile is the percentage of all fertile eggs that were incubated. Because infertility occurred prior to the eggs being layed, infertility could not have been affected by the treatment. Both Percent Hatch and Hatch of Fertile were higher in the treated egg group as compared to control eggs. A difference of 3 percentage points in Hatch of Fertile would be a large improvement in embryonic survival by poultry industry standards.

Embryonic "Pipped" eggs are a hatching failure where the chick initially breaks through the eggshell but is unable to complete hatching. "Early Dead" refers to embryos that died before 8 days of incubation. "Mid-Dead" are embryos that died between 8 and 16 days of incubation. "Late Dead" are embryos that died after 16 days of incubation but before pipping the eggshell. "Contaminated" eggs had obvious bacterial overgrowth. "Mold" refers to eggs with visible mold growth on the shell membrane (see attached photo). The number of pipped eggs was lower in the treated group. The number of early dead embryos was lower in the treated eggs and was the number of eggs contaminated by mold. These results are consistent with a lower microbiological load on the eggs during storage and incubation.

Table 1.

Treatment	Total Set	Number Hatched	Percent Hatched	Hatch of Fertile	Percent Unhatched						
					Pip	Infertile	Early Dead	Mid Dead	Late Dead	Contaminated	Mold
Treated	562	507	90	95	0.18	4.98	1.25	0.00	2.31	1.07	0.00
Control	571	507	89	92	0.53	3.85	2.98	0.00	2.45	1.05	0.35

Chick Survival and Growth at One Week Post-Hatch: Survival of chicks during the first 7 days post-hatching is widely used in the poultry industry as a combined indicator of the quality of chicks coming from the hatchery and husbandry at the broiler farm. Chick mortality (Table 2) during the first 7 days following hatching was reduced by almost half in the chicks hatched from the treated eggs. Doubling 7-day chick survival would be a very large improvement by poultry industry standards. Body weight at the end of 7 days was slightly higher in the control chicks. Longer duration studies would be required to determine whether this difference in body weight would persist for longer than 7 days.

Table 2.

Treatment	7 day % mortality	Day 0 body weight (g)	7 day body weight (g)
Treated	0.015	44	300
Control	0.028	43	311

Trial 2

Hatching Results and Residue Analysis: Results for hatching and residue analysis are presented in Table 3. Hatch of fertile was somewhat less than in Study 1 as expected for eggs from an older flock of hens. However, as in Study 1, both Percent Hatch and Hatch of Fertile were higher in the treated egg group as compared to control eggs.

"Cull" chicks were recorded in Trial 2. Cull chicks are chicks that have hatched but had defects rendering them unusable. Treatment reduced the number of cull chicks, as well as reducing the number of pips, early deads, and late deads, and contaminated embryos. Mid-dead chicks were higher in the treated group.

Table 3.

Treatment	Total Eggs Set	Number Hatched	Percent Hatch	Hatch of Fertile	% Cull	%PIP	% Infertile	% Early	% Mid	% Late	% Contaminate	MOLD
Treated	1909.00	1696.00	88.80	91.20	0.34	0.63	3.42	1.82	1.37	1.48	0.11	0.00
Control	1985.00	1712.00	86.20	88.40	1.56	1.67	2.44	1.94	0.67	3.11	0.44	0.00

Chick Survival and Growth at One Week Post-Hatch: Seven-day chick survival data and growth results are presented in Table 4. As in Study 1, treated chicks had 7-day mortality rates at about half that of control chicks. Unlike Study 1, the treated chicks also were slightly heavier than controls at 7 days of age.

Table 4.

Treatment	7 day % mortality	Day 0 body weight (g)	7 day body weight (g)
Treated	0.0075	42.4	196
Control	0.0300	43.1	184

Summary and Conclusions

Embryonic survival was increased and microbiological contamination was decreased by the sanitation treatment in both studies. By broiler industry standards, the increases seen for "hatch of fertile" were large. Reductions in embryonic mortality were also relatively large.

Improved survivability of the chicks for the first 7 days post-hatch is used by the broiler industry as an index of both initial chick quality and initial husbandry in the poultry house. The increased survivability (reduced mortality) see in both Study 1 and Study 2 would be considered very large if replicated in commercial poultry houses.

Study 1 did not find an increase in chick growth by 7 days post-hatch. However, an increase in the body weight of chicks from treated eggs was seen in Study 2. Study 1 eggs were from younger hens at peak egg production. Initial chick quality is generally best from eggs from hens at peak. Because of this, the chicks in Study 1 were likely to be close to optimum condition at hatch, so there was not much improvement to be had. Eggs from Study 2 were from older hens with poorer egg shell quality with lower fertility, and likely carried a higher microbiological load. It would be expected that embryos and chicks from these eggs would have obtained relatively more benefit from the sanitation treatment than the eggs from Study 1 and explaining the apparent improvement in growth seen in Study 2.

In conclusion, the sanitation treatment used in these studies appears to have no adverse effects on broiler hatching eggs and the results are consistent with increased embryonic survival and reduced chick mortality post-hatch.

Mold in hatching eggs following in-ovo vaccination