Preincubation Dipping of Turkey Hatching Eggs

1. Effect of Shell Treatment

on Amount and Variability of Fluid Intake

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SUMMARY

Two techniques for egg-shell treatment were evaluated for their effects on the quantity and variability of the fluid absorbed by turkey hatching eggs subjected to the temperature-differential method of egg dipping.

The use of a pin or drill to pierce a uniform hole through the shell and membrane(s) on the large end of each egg caused a significant increase in the quantity of dip solution absorbed. Variability in fluid uptake by the eggs also was reduced significantly.

Utilization of dilute acid to remove cuticle completely from the whole egg surface or partially from either end also had similar effects.

The techniques described also permit an effective dipping procedure that does not require the preheating step.

INTRODUCTION

Antibiotic treatment of eggs by the temperature-differential method has been used widely for controlling certain egg-transmitted bacterial infections (3,5,7,8,11,16). Individual eggs differ in the volume of fluid they can absorb in this procedure (1,7). This variability, attributed mainly to the unequal number of patent shell pores caused by uneven cuticle deposition on individual eggs (2), makes it impossible to deliver a uniform effective dose into

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Fluid intake of dipped eggs

Each egg, thus limiting the usefulness of the technique in disease control.

Alls et al. (1) established the influence of dipping time, temperature-differential, and other factors on the quantity of antibiotic that enters dipped eggs. Newman (11) demonstrated that fluid intake was increased by dipping each egg twice. Alls et al. (2) showed that complete removal of the shell cuticle with dilute acid enhanced shell permeability, while McCapes’ experiment with small groups of eggs (unpublished data) indicated that piercing the shell at either end before dipping might give more uniform fluid absorption.

The investigations reported here were conducted to examine the effects of various shell treatments on the amount and uniformity of antibiotic solution absorbed by eggs dipped before incubation.

MATERIALS AND METHODS

Hatching eggs. The four trials involved 1,480 eggs. Eggs in trials 1 and 2 were from the Rose-A-Linda Turkey Breeding Farms, Inc., of Elberta, California. They were sanitized with a solution of quaternary ammonium compound (QAC) in a commercial brush washing machine (Magic egg washer, National Poultry Equip. Co.) before delivery to the hatchery. Eggs in trials 3 and 4, from the Nicholas Turkey Breeding Farms, Inc., of Sonoma, California, were cleaned with steel wool and fumigated with formaldehyde.

All the eggs in each trial were from the same breeder source and had been harvested on the same day. Among trials, the length of time between harvesting and shell treatment varied from 4 to 10 days.

Shell treatment. Two types of shell treatment were tested: 1) piercing of uniform holes in the egg shell with a pin or drill; and 2) immersion of the egg in dilute acid to remove cuticle.

1) Drilling. The drilling site, at the center of the large end, was disinfected with merthiolate. A single hole was drilled through the shell and underlying outer membrane with a dental drill fitted with a 1.0–1.2 mm burr. After the egg was dipped and reweighed the hole was sealed with Duco cement (E. I. du Pont de Nemours and Co.).

2) Pinhole. After disinfection as above, the shell and underlying membranes at the center of the large end were pierced with a 1/32 × 1 inch sterile pin. The hole was sealed as above.

3) Acid immersion. Different concentrations of hydrochloric
Table 1. Experimental design and results of trial 1: the effect of a hole drilled on the large end, preheating in hot water, and dipping time on fluid intake by turkey hatching eggs dipped in tylosin solution by the temperature-differential method.

<table>
<thead>
<tr>
<th>Dipping time:</th>
<th>0 minutes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2.5 minutes</th>
<th>5.0 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat treatment:</td>
<td>Heated</td>
<td>Not heated</td>
<td>Heated</td>
</tr>
<tr>
<td>Egg treatment:</td>
<td>Drilled</td>
<td>No hole</td>
<td>Drilled</td>
</tr>
<tr>
<td>Group no.:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mean (g):</td>
<td>-0.04</td>
<td>-0.11</td>
<td>-0.07</td>
</tr>
<tr>
<td>Standard deviation:</td>
<td>±0.15</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td>Range (g):</td>
<td>0.79</td>
<td>0.08</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<sup>a</sup>Eggs in these groups were not dipped in cold antibiotic solution.
<sup>b</sup>40 eggs per group.
<sup>c</sup>Data bearing different letters differ significantly (P = 0.01).
<sup>d</sup>Data bearing same letters were compared and found to differ significantly (P < 0.01).
<sup>e</sup>Broken egg.

Table 2. Experimental design and results of trial 2: effect of dipping time, and complete immersion in 0.5N hydrochloric acid for various periods, on the fluid intake by turkey hatching eggs heated in hot water before dipping in cold tylosin solution for 5 or 10 minutes.

<table>
<thead>
<tr>
<th>Acid treatment time:</th>
<th>0 sec&lt;sup&gt;f&lt;/sup&gt;</th>
<th>15 sec</th>
<th>30 sec</th>
<th>60 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipping time:</td>
<td>5 min</td>
<td>10 min</td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Group no.:</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Mean fluid&lt;sup&gt;c&lt;/sup&gt; intake (g):</td>
<td>0.41n</td>
<td>0.50n</td>
<td>0.73p</td>
<td>0.89q</td>
</tr>
<tr>
<td>Standard deviation:</td>
<td>±0.23r</td>
<td>±0.26r</td>
<td>±0.17su</td>
<td>±0.09st</td>
</tr>
<tr>
<td>Range (g):</td>
<td>0.19-0.89</td>
<td>0.06-1.07</td>
<td>0.98-1.23</td>
<td>0.69-1.14</td>
</tr>
</tbody>
</table>

<sup>f</sup>Eggs in groups A and B were not treated with acid.
<sup>c</sup>45 eggs per group.
<sup>d</sup>Data with different letters differ significantly (P = 0.01).
<sup>e</sup>dr and s differ; t and u also differ significantly (P < 0.01).
acid were tried and 0.5N was found to be adequate for removing cuticle from the shell. The HCl was applied by egg immersion to either the entire shell surface or the large or small end. Afterwards, the egg was immediately rinsed in running tap water.

**Egg-dipping procedure.** The temperature-differential egg-dipping procedures followed were those used at two commercial hatcheries: 1) preheating eggs for 10 minutes in a solution of QAC (500 ppm) held at 42 C before immersing them fully in a cold (7.8–9 C) aqueous solution containing 3,000 ppm tylosin tartrate, 500 ppm QAC, and 0.003% sodium nitrite. The sodium nitrite, added by Rose-A-Linda hatchery on the advice of another commercial hatchery, was supposed to counteract any adverse effects arising from the use of galvanized-iron dipping trays; 2) preheating for 3 hours in hot (36 C) circulating air before dipping in a cold (4.4–5.6 C) aqueous solution containing about 800 ppm gentamicin sulfate (American Scientific Labs) and 250 ppm QAC.

All eggs were kept at room temperature (20–24 C) for at least 2 hours while being prepared for dipping.

**Egg identification and weighing.** Each egg was numbered, and weighed to the nearest 0.01 gram on a Mettler P1200/P1200N scale before and after dipping.

**Trial 1.** This was designed as a $3 \times 2 \times 2$ factorial experiment. The 480 eggs involved were randomly (14) assigned to 12 groups of 40 eggs each as shown in Table 1. Drilled and undrilled eggs in groups 1, 2, 5, 6, 9, and 10 were heated in hot water, allowed to drain for 1 minute and then dipped for 0, 2.5, or 5 minutes in cold tylosin solution. The other six corresponding groups were not preheated.

**Trial 2.** The 360 eggs used in this trial, which was designed as a $4 \times 2$ factorial experiment, were randomly assigned to 8 groups of 45 eggs each (Table 2). Eggs in groups A and B, C and D, E and F, and G and H were completely immersed in HCl for 0, 15, 30, and 60 seconds, respectively. They were then rinsed, heated in hot water, and allowed to drain for 1 minute before dipping for 5 or 10 minutes.

**Trial 3.** This trial involved 320 eggs which were randomized into 4 groups of 80 eggs each (Table 3). One group had a pinhole pierced into the shell at the large end of each egg while two other groups had one end or the other immersed in acid for 30 seconds. The fourth group consisted of untreated controls. Acid treatment of the small or large end was confined to about 1/3 of
Table 3. Experimental design and results of trials 3 and 4: effect of piercing a hole on the large end with a pin, or immersing the large or small end of the egg in 0.5N hydrochloric acid for 30 seconds, on fluid intake (g) by turkey hatching eggs dipped for 2.5 or 10 minutes in gentamicin solution by the temperature-differential method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pinhole on large end</th>
<th>Acid treatment small end</th>
<th>Acid treatment big end</th>
<th>Eggs with untreated shells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 3: dipped 2.5 min after 3 hr in hot circulating air.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean fluid intake (g)</td>
<td>0.31d</td>
<td>0.34d</td>
<td>0.32d</td>
<td>0.15e</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.05f</td>
<td>±0.09k</td>
<td>±0.09h</td>
<td>±0.10h</td>
</tr>
<tr>
<td>Range</td>
<td>0.15–0.47</td>
<td>0.00–0.52</td>
<td>0.14–0.53</td>
<td>-0.03–0.41</td>
</tr>
<tr>
<td>Trial 4: dipped 10 min without preheating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean fluid intake (g)</td>
<td>0.46l</td>
<td>0.61k</td>
<td>0.48m</td>
<td>0.43l</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.08n</td>
<td>±0.07n</td>
<td>±0.06n</td>
<td>±0.15p</td>
</tr>
<tr>
<td>Range</td>
<td>0.16–0.63</td>
<td>0.44–0.78</td>
<td>0.30–0.63</td>
<td>0.08–1.13</td>
</tr>
</tbody>
</table>

*Each trial consisted of 4 groups of 80 eggs each.

*Cuticle removed from about 1/8 length of egg at the big end in trial 4 but from about 1/3 in the other three acid-treated groups.

*Data bearing different letters differ significantly (P ≤ 0.01).

the egg’s length. The eggs were heated for 3 hours in hot circulating air before being dipped for 2.5 minutes in cold gentamicin solution.

**Trial 4.** The experimental design and treatment of eggs were identical to those used in trial 3. However, in trial 4, the eggs were not preheated before dipping for 10 minutes and, in the group of eggs treated with acid at the large end, cuticle was removed from about 1/8 of the egg’s length.

**Statistical analysis.** The factorial-design experiments were evaluated by the analysis of variance (14). Other statistical tests included Duncan’s multiple-range test (15) and Cochran’s and Bartlett’s tests for homogeneity of variances (4).

**RESULTS**

Untreated eggs lost an average of 0.09 g during trial 1 (Table 1, group 4). This is consistent with the findings, in our preliminary trials, that normal turkey hatching eggs kept at room temperature (20–24 C) lost a mean of 0.19 ± 0.08 g over 24 hours, and indicates that the reported fluid intake of dipped eggs is probably a little less than actual intake since a minimum of 3 hours elapsed between initial and final egg weighing.

As shown in Table 1, fluid intake varied significantly less (P<0.01) in drilled than in undrilled eggs. Also, the quantity of fluid absorbed by dipped eggs was significantly influenced (P<0.01) by each of the three components of treatment: a) preheating
in hot water; b) duration of dipping in cold solution (dipping time); and c) a drilled hole. There was a significant positive linear component and a significant negative quadratic component in the sum of squares of dipping time (P<0.01) indicating that the rate of increase in fluid absorption falls off as dipping time is increased.

Table 2 shows that HCl treatment of whole shells in trial 2 significantly (P < 0.01) reduced variations in intake of cold tylosin solution by dipped eggs. The reduction in variation was greatest in the group of eggs dipped for 10 minutes after immersion in acid for 15 seconds. The amount of fluid absorbed was influenced significantly (P<0.01) by each of the two components of treatment: 1) acid treatment; and 2) dipping time. The rate of fluid intake increased with acid treatment time and then declined.

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**Fig. 1.** Distribution of gentamicin solution intake within differently treated groups of turkey hatching eggs (80 eggs per group) dipped for 2.5 minutes after preheating in hot air for 3 hr.
Table 3, trial 3, shows the mean fluid intake by untreated controls and the three groups of eggs whose shells were pierced on the large end with a pin or partially (1/3 egg's length) immersed in acid at either end. Fig. 1 illustrates fluid intake distribution among individuals in the four groups. When all four groups were considered, Bartlett's test showed that at least two of the variations from the means differed significantly (P < 0.01). When the data for the eggs with a pinhole were not used in the analysis, the differences were no longer significant. The analysis of variance indicated a significant difference among the values for the population mean fluid intake by eggs in the four groups (P < 0.01). Duncan's multiple-range test established that the control differed from each of the experimental groups, but that the experimental groups did not differ significantly among themselves.

Table 3, trial 4, shows the mean fluid intake by groups of eggs treated exactly like those in trial 3 except that they were not preheated prior to dipping, and acid treatment of the large end was confined to 1/8 of the egg's length. The variation in the quantity of cold antibiotic solution absorbed by eggs in each of the three treated groups was significantly less than that of the controls (P < 0.01). The value for the population mean fluid intake by the eggs immersed in acid at the small end was significantly larger than that of any of the other three groups, while the intake by those de-cuticled at the large ends was also significantly larger than that of eggs with the pinhole or the controls (P = 0.01). The values for the latter two groups did not differ from each other.

DISCUSSION

The development of a practical method delivering a uniform and effective dose of antibiotic into each egg is basic to achieving control of egg-transmitted bacterial infections of poultry. Antibiotics administered by egg dipping usually reduce but do not eliminate egg contamination (5,6,16). Egg injection has been reported (13,17) as successful in treating populations of chicken eggs against Mycoplasma gallisepticum. Hofstad (6) and McCapes et al. (10), using different inoculation sites, investigated the technique for use in controlling Mycoplasma meleagrisidis in turkeys. More recent investigations by McCapes et al. (9) indicate that inoculation of effective but nontoxic levels of antibiotic through the small ends of eggs is adaptable for use on a large scale.
Fluid intake of dipped eggs

Nationwide eradication of egg-transmitted infections such as salmonellosis, arizonosis, or mycoplasmosis through antibiotic therapy of eggs would involve treating very large numbers in different hatcheries and basic breeding production units, compounding mechanical and human errors inherent in an egg-injection procedure and causing eradication to fail just because a single infected egg was not inoculated. In addition, injection of antibiotics would probably be most effective against organisms located in the interior of eggs, less so against those associated with the shell membranes, and ineffective against any in and on the shell. The chance of error can be substantially reduced if a uniform dose could be attained in an egg-dipping process. Moreover, dipping ensures contact between the antibiotic solution and virtually all parts of the egg including the entire shell surface, the patent shell pores and adjacent membranes, albumen, yolk, and the germ cell (2). This is very important from the standpoint of concurrent eradication of more than a single infectious agent, since different types of microorganisms have a predilection toward different sites in an egg.

Our results agree with reports by Alls et al. (1) and Newman (11) on the influence of dipping time and temperature-differential on the quantity of dip solution absorbed. They further establish that drilling or piercing uniform holes in the large ends of turkey hatching eggs increases the quantity of dip solution absorbed and significantly reduces variability in intake. The similar effects from complete removal of cuticle by dilute acid also agree with results of Alls et al. (2), who used a different analytical approach. Removal of about one-third or less of cuticle from either the small or large end of eggs gave effects akin to those due to complete removal.

Twelve-hundredths grams of 1000 ppm gentamicin solution is equivalent to 0.11 mg of gentamicin sulfate. That has been found adequate for preventing air-sac lesions in day-old pouls hatching from eggs injected before incubation (10). As shown in Table 3, trial 4, the smallest quantity of gentamicin solution absorbed by an egg in any of the treated groups is 0.16 g, and that was by one of the eggs dipped without preheating. That indicates that the shell-treatment techniques studied can also enable the absorption of uniform and potentially effective quantities of dip solution by eggs immersed directly in cold antibiotic solution, thus eliminating the need for preheating above room temperature. Current in-
vestigations are determining the effects of the various shell treatments on hatchability. Preliminary results suggest that piercing a hole on the large end with a pin might reduce hatchability considerably.

REFERENCES


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