Comparison of Virkon S® and Formaldehyde on hatchability and survival rate of chicks in disinfection of fertile eggs

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SUMMARY

For comparison of Virkon S® and formaldehyde on hatchability, survival rate and microbial population, 120 fertile eggs from broiler breeder farms were collected and divided into 4 equal groups. In group 1 and 2, all eggs were sprayed with Virkon S® in 1/100 and 1/200 dilutions, respectively. In group 3, the eggs were disinfected with formaldehyde and in group 4, the eggs were sprayed with sterile water, as negative control. All eggs were incubated for 21 days and assayed hatchability rate. After hatching, the chicks were monitored for 7 days and the survival rate was recorded. Furthermore, the contamination of non-hatched embryo was examined for Escherichia coli. The eggs that were disinfected with 1/100 dilution of Virkon S® had lower hatchability and growth indices but not in 1/200. It seems Virkon S® can be a good alternative for disinfection of fertile eggs with proper concentration and condition.

Key words: Escherichia coli, Formaldehyde, Hatchability, Virkon S®

RESUME

Comparaison du Virkon S® et du formaldéhyde sur le taux d’éclosion et le taux de survie des poussins après désinfection des œufs

Cent vingt œufs fertiles provenant de fermes d’élevage de poulets ont été collectés et divisés en 4 groupes égaux. Dans le groupe 1 et 2, tous les œufs ont été pulvérisés avec du Virkon S® aux dilutions respectives de 1/100 et 1/200. Les œufs du groupe 3 ont été désinfectés avec du formaldehyde alors que les œufs du groupe 4 ont été pulvérisés avec de l’eau stérile. Tous les œufs ont été incubés pendant 21 jours et le taux d’éclosion a été enregistré. Après l’éclosion, le taux de survie des poussins a été mesuré pendant 7 jours. La contamination par Escherichia coli des embryons non éclos a été examinée. Les œufs désinfectés par une solution de Virkon S® à 1/100 avaient un taux d’éclosion inférieur et les indices de croissance des poussins étaient détériorés par rapport aux autres groupes, ces effets n’étant plus observés à la dilution de 1/200.

Mots-clés Fr: Escherichia coli, formaldéhyde, Virkon S®, éclosabilité

Introduction

Avian colibacillosis is an infectious disease of birds caused by Escherichia coli. It is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry. Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages. This disease has an important economic impact on poultry production worldwide. The majority of economic losses of this disease are mortality and decreasing in productivity of the affected birds [12]. Fecal contamination of egg may result in the penetration of E. coli through the eggshell and may spread to the chickens during hatching. It is often associated with high mortality rates, or it may give rise to yolk sac infection [10]. Fecal contamination of the eggshell is possible during the passage of the egg through the cloaca after laying. The latter possibility is considered as the main route of infection for the egg [11]. Before hatching, E. coli causes yolk sac infection and embryo mortality. The chick can also be infected during or shortly after hatching. In these cases, retained infected yolk, omphalitis, septicemia and mortality of the young chicks up to three weeks of age is seen [11]. A first step is the prevention of egg contamination by fumigating them within two hours after lay, and by removing cracked eggs or eggs soiled with fecal material [10]. However, the contamination of egg shells is inevitable. Therefore, disinfection of fertile egg before setting in hatchery is necessary to reducing egg contamination. Hence, it is tried in this investigation to evaluate the efficiency of a widely spectrum disinfectant (Virkon S®) with commercial conditions (using of formaldehyde gas) in disinfecting surface of eggs, and the biological characteristics of fertile eggs such as the rate of hatchability, survival of hatched chicks, microbial culture of the chicks and probably losses of chicks.

Materials and methods

120 fertile eggs are prepared from Behjoujeh Zagros Aviculture (Chaharmahal-va-Bakhtiyari province, Iran) to compare the efficacy of the Virkon S® and formaldehyde gas in decreasing bacterial contamination of eggshells from fertile eggs. The eggs were disinfected primary in the breeder farm (2 hours after laying) and the one-week were stored in the refrigerator and then transferred to microbiological
laboratory. We divide 120 eggs into four groups (with 3 replicates). In the first group, fertile eggs were disinfected with Virkon S® with 1/100 dilution. In the second group, the fertile eggs were disinfected with Virkin S® with 1/200 dilution. In the third group, the fertile eggs were disinfected with formaldehyde gas (by conventional commercial method). In the fourth group, the fertile eggs were sprayed with distilled water as negative control.

Virkon S® (Dupont co., USA) is a wide commercial disinfectant, being effective against viruses, bacteria and fungi. This material is in the form of pink powder, easily solved in water, providing a uniform and transparent substance with the same color. Virkon S® is a balanced, stabilized blend of peroxygen compounds, surfactant, organic acids and inorganic acid.

In the current study, Virkon S® was used with the dose of 1500 ppm, and to provide the dose, the prepared solution (in the volume of one ml) is sprayed on each egg. After spraying, the fertile eggs were remained in room temperature for drying. The eggs that are exposed to formaldehyde gas were disinfected in the incubator, with the usual dose and method (with commercial conditions). Then, in aseptic conditions, the eggs were incubated and the rate of hatching eggs is considered in the different groups. Moreover, the rate of survival of the hatched chicks in the first week of life and bacteriology culturing [2] from the death embryos were analyzed.

All the fertile eggs were incubated for 18 days with the temperature of 37.5°C and 55% of relative humidity, with minimum 4 daily turning in the vertical position. After 18 days of incubation, the fertile eggs were placed horizontally in the hatchery tray, being incubated without turning in 36.5°C and 70% of relative humidity. After hatching, the chicks were taken out of the incubator, and kept in 32-33°C with free access to water and feed. The rate of hatching chicks from the fertile eggs was recorded at this stage, for each group.

After the end of the 22nd day, the fertile eggs that were not hatched were opened and the yolk sac or the heart was sampled for bacterial cultures. The chicks were taken under the care for 7 days, and the rate of survival of the chicks in the groups was analyzed and compared.

The chicks that died in first 7 days after hatching were examined bacteriologically by culturing of the yolk or heart. At the end of 7 days old, the weight gain, rate of consuming feed and Feed conversion rate (FCR) in different groups were calculated.

Sigma State 2.0 software was used to analyze the data. Data was analyzed with One way ANOVA for investigation of significant differences between the different groups. If the difference was between groups, it was evaluated by Tukey test. The significant difference level for was considered to be P<0.05.

### Results

#### RATE OF HATCHABILITY

Comparison of the rate of hatching in the groups receiving disinfectants and the control group shows that using formaldehyde and Virkon S® (1/200 dilution) have no effects on hatching of the eggs, while using Virkon S® (1/100 dilution) could significantly reduce the rate of hatching of fertile eggs for broilers (P=0.002). (Table I).

#### SURVIVAL RATE

Comparison of the affected embryos by E. coli in different groups receiving disinfectants shows that using formaldehyde, Virkon S® (1/100) and Virkon S® (1/200) could significantly reduce the contamination to E. coli of the lost embryos (reliability levels of 0.18%, 0.12% and 0.33%, respectively). In other words, it is shown in a general comparison that there is a significant relation between using disinfectants and reducing infection of the embryos (P=0.005) (Table II).

Comparison of the survival rate of hatching chicks in different groups receiving disinfectants shows that using formaldehyde, Virkon S® (1/100) and Virkon S® (1/200) could significantly reduce the rate of hatching of the eggs, while using Virkon S® (1/200 dilution) have no effects on hatching of the eggs, while using Virkon S® (1/100 dilution) could significantly reduce the rate of hatching of fertile eggs for broilers (P<0.001) (Table III).

#### GROWTH INDICES

Results show that the weights of hatching chicks in the group disinfected by formaldehyde are significantly higher than the groups receiving Virkon S® (1/200) (P<0.05), while no significant difference exists between the other groups.

Comparison of weights and feedings by the end of the first week show that the group receiving formaldehyde or Virkon S® (1/100) had higher rates in weight and feeding. Thus, statistically, a significant difference exists between the groups that were disinfected by formaldehyde or Virkon S® (1/100) with other groups, while no significant difference is observed between these two groups.

Comparison of the FCR at the end of the first week of age shows that no significant difference exists between these groups. (Table IV).

### Discussion

There are many pathogens that can infect fertile eggs comprising *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Colostrum*, *Bacillus cereus*, *Salmonella typhimurium* and *Enterococcus*, but the main pathogen that
Affects fertile eggs more than others is usually *Escherichia coli* [15]. This pathogen could enter the egg from the pores on the eggshell after contamination of the egg by feces. There are about 7000-17000 pores on an eggshell that provide a proper place for entering the bacteria [17, 20]. On the other hand, there usually are about 100000 microorganisms on the surface of a clean eggshell [9], indicating necessary cleaning and disinfecting the fertile eggs for hatching. Furthermore, it is necessary to cleaning and washing the hatchery. The methods such as fumigation, spraying or in some cases applying UV radiation are usually used for disinfecting the hatchery and the fertile eggs [6]. About 80-85% of the infections are reduced by washing the hatchery, while the remaining microorganisms are eliminated by disinfecting. According to HACCP standards, disinfectants reduce about 99.99% of the microbes. However, not disinfecting the fertile eggs infects the embryos to *E. coli*, causing losses during embryonic stage or even the first week of growing. Thus, not disinfecting or improper disinfecting causes the growth of bacteria and hatching of low quality chicks as well as inappropriate performance of these chicks [16], and eventually increases losses during the first week of breeding, due to infection of the yolk sac [13].

Until now, the main and most effective disinfectant for reducing the contamination of fertile eggs is formaldehyde gas. Although formaldehyde is a super antimicrobial element and it is cheaper and more accessible as compared to other disinfectants, but if it is used improperly, it could lead to the death of embryos. Moreover, formaldehyde is a respiratory

<table>
<thead>
<tr>
<th>Condition</th>
<th>Groups</th>
<th>Control</th>
<th>Formaldehyde</th>
<th>Virkon S* (1/100 dilution)</th>
<th>Virkon S* (1/200 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of hatched chicks</td>
<td>27</td>
<td>25</td>
<td>15</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>No. of chicks not hatched</td>
<td>3</td>
<td>5</td>
<td>15</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Percentage of hatching</td>
<td>90</td>
<td>83.33</td>
<td>50</td>
<td>76.6</td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>0.70</td>
<td>0.002</td>
<td>0.29</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Table I: Relation between using disinfectant substances and the rate of hatching eggs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Groups</th>
<th>Control</th>
<th>Formaldehyde</th>
<th>Virkon S* (1/100 dilution)</th>
<th>Virkon S* (1/200 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost embryos that were affected by <em>E. coli</em></td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lost embryos that were not affected by <em>E. coli</em></td>
<td>0</td>
<td>5</td>
<td>13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>0.018</td>
<td>0.012</td>
<td>0.033</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Table II: Relation between using disinfectant substances and the rate of affected infection in lost embryos in hatchery

<table>
<thead>
<tr>
<th>Condition</th>
<th>Groups</th>
<th>Control</th>
<th>Formaldehyde</th>
<th>Virkon S* (1/100 dilution)</th>
<th>Virkon S* (1/200 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of remaining chicks at the end of 1st week</td>
<td>14</td>
<td>24</td>
<td>14</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>No. of dead chicks at the end of 1st week</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Percentage of chicks surviving at the end of 1st week</td>
<td>51.85</td>
<td>96</td>
<td>93.33</td>
<td>95.65</td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>*</td>
<td>0.001</td>
<td>0.007</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Table III: Relation between disinfectant substances and the rate of first week losses in chicks

<table>
<thead>
<tr>
<th>Component</th>
<th>Groups</th>
<th>Control</th>
<th>Formaldehyde</th>
<th>Virkon S* (1/100 dilution)</th>
<th>Virkon S* (1/200 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight in 1st day</td>
<td>47.0+3.10a</td>
<td>50+2.85a</td>
<td>47.7+3.26ab</td>
<td>45.0+3.00a</td>
<td></td>
</tr>
<tr>
<td>Weight in 7th day</td>
<td>138+4.69c</td>
<td>147+5.16c</td>
<td>136+4.02c</td>
<td>145+4.39c</td>
<td></td>
</tr>
<tr>
<td>Feed consumption in the 1st week</td>
<td>112+5.98bc</td>
<td>120+8.30bc</td>
<td>110+6.21bc</td>
<td>117+7.45bc</td>
<td></td>
</tr>
<tr>
<td>FCR at the end of 1st week</td>
<td>0.81+0.94a</td>
<td>0.82+0.03a</td>
<td>0.81+0.07a</td>
<td>0.80+0.05a</td>
<td></td>
</tr>
</tbody>
</table>

*a,b,c*: Different superscripts in each row shows the significant difference between the groups (P<0.05).

Table IV: Comparison of the components of growth in different groups
stimulant that is harmful to humans. Due to the links of this composition to proteins, nucleic acids and unsaturated fatty acids it can denaturate of proteins. Also, formaldehyde has toxic effects and due to cytotoxic effects, it causes inflammatory reactions, necrosis and mutation effects and using of this material can cause cancer in humans [18].

Thus, as a general principle, disinfectant compositions should have wide spectral effects and minimal biological effects. Hence, in the recent study, special considerations are given to using the products that could be a proper replacement for formaldehyde gas. The present study deals with analyzing the effects of Virkon S® as a wide spectral disinfectant with high reliability [3] in disinfecting fertile eggs in comparison to formaldehyde gas.

The results of this study show that disinfecting fertile eggs could reduce the infections of embryos, reduce the first week losses and increase the weight and feeding during the first week, while it has no effects on hatching and FCR at the end of the first week.

Comparison of hatching rate in different groups shows that the lowest rate belongs to the group using Virkon S® (1/100 dilution), and the difference in hatching rate as compared to other groups is significant. The reason that all the receiving groups of disinfectants could improve hatching of chicks, but using Virkon S® (1/100 dilution) has reduced it may be due to toxic effects of the high dose of this composition on the primary embryo, since spraying the disinfectant on fertile eggs causes the penetration of this composition from the pores of the eggshells into the egg content, that provided toxic effect on the embryo.

Investigations on the effects of some disinfectants with different effective substances, including the ordinary method of using formaldehyde, combination of bleach and an organic acid and chloride derivatives, on the rate of hatching, and showed that disinfectants with different effective substances have no considerable effects on the rate of hatching [5]. Also disinfecting of fertile eggs with hydrogen peroxide has no effects on the rate of hatching [14]. Although the results of these studies are concurrent with the recent research about the ineffectiveness of disinfectants on the rate of hatching, but there is another report shows that some disinfecting substances could reduce hatching. The hatching rate of fertile eggs that confront with disinfectants is 6% less than the fertile eggs that are not disinfected [2]. Comparison of the average of the hatching rates of the recent study shows that disinfecting the eggs with formaldehyde reduces 6.6% hatchability while disinfecting with Virkon S® (with 1/200 and 1/100 dilution) could reduce 13.4% and even 50% hatchability. However, it seems that the disinfectant compositions, dilution and the duration of exposure are important and effect on outcome of hatchability. In this regard, the hatching rate of the eggs disinfected by UV radiation or negative ions was relatively more than the ordinary method of formaldehyde gas [8]. Moreover, ozone has better effects on hatching than formaldehyde (91.79% against 91.10%) [7].

Regarding to the 6.6% reduction in hatchability in the group receiving formaldehyde, it seems that gaseous or spray disinfectants could have biocide effects and be rather toxic for the embryos. Thus, the replacement of formaldehyde by safety biologic compound for human health and environmental health care is necessary.

The results of recent study show that disinfectants by reducing the rate of infection of fertile eggs have great roles in promoting health and survival of hatching chicks. The eggshell contamination with feces provides a suitable place for growth of bacteria [4, 5, 19]. Analysis of the effects of disinfectants on the population of bacteria showed that different compositions of disinfectants have negative effects on the total population of the bacteria [1, 5], such that the rate of infection of an egg affect the rate of survival and growth of the chicks. There is a study that has dealt with the effect of hydrogen peroxide in the form of fumigation on the survival and growth indices. It showed that using hydrogen peroxide as a disinfectant of fertile eggs has no effect on survival of chicks, their weight and also the feed conversion rate (FCR), but increase the duration of survival of the yolk sacs in the hatched chicks [14].

The results of this study are in conformity with the results of other studies about the lack of affect ability of the FCR, but regarding the effects of disinfecting on the body weight and food intake, the recent study indicates that disinfecting with formaldehyde and Virkon S® (1/200 dilution) could improve the body mass index and food intake during the first week, and the improvement may be due to reducing the contamination of the eggshell and reducing the events for infecting the yolk sac.

No studies have yet been done on using Virkon S® as a substitute for formaldehyde gas in hatchery. In the recent research, as a basic study, it was shown that Virkon S® with 1/200 dilution could compete with formaldehyde in the investigated parameters in this study. However, regarding the sensitivity and importance of disinfecting fertile eggs, it is necessary to provide more extensive studies, considering the growth factors and rate of survival, and also the analysis of metabolic indices such as sensitivity to ascites and others.

References

EFFECT OF DISINFECTANTS ON FERTILE EGGS


