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Impact of Boiler Breeders Hatching Eggs Disinfection Time on Some Hatchability Parameters

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Abstract

(Control of microorganisms on hatching eggshell surface requires an effective disinfectant to kill the pathogens without injury to the live chick embryo. The present experiment was carried out to study the effect of hatching egg sanitization time by different disinfectants on some hatchability parameters. A total of 600 broiler breeder fertile eggs from a commercial broiler strain (Saso) aged 35 wk were reared on a deep litter system at a private farm in Gharbiya Governorate, Egypt. All eggs were randomly divided into three treatment groups of 200 eggs each. The eggs in the first group were kept as a control without treatment. While, the eggs in the second and third groups were subdivided into equal four subgroups treated by Propolis 14%, Ethyl alcohol 70%, TH4 0.5% and Virkon S 0.5 %., respectively, which, they disinfected before 6hrs from egg laying in the second group and disinfected after 6hrs from egg laying in the third group. Results revealed that there was no significant difference in egg weight loss during egg storage period and hatch time in relation to time of egg disinfectants (before and after 6 hrs from egg laying) and among different disinfectants used. But, the hatchability % and TBC were reduced by using propolis 14% as egg disinfectant.

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On the other side, total embryonic mortality % and chick hatch weight were increased by application of propolis 14% as egg disinfectant in compared to other disinfectants used. In conclusion, the application of egg sanitization process immediately after egg laying was beneficial in lowering TBC. Using propolis 14% in the disinfection process reduced the egg weight loss and hatchability percent but increased the hatching chick weight and chick weight on day 7 of growing period).

Keywords: propolis; bacterial count; hatch time; hatchability; embryonic mortality.

1. Introduction

Poultry industry and broilers chickens are considered one of the important sources of meat due to its advantage of fast growing birds in less than 45 days. Hatching eggs are very important sources for young chicks which grow to become chicken. The ideal environment for the embryo development is the same needed for microorganism multiplication. Therefore, contaminated eggs will disseminate microorganisms in incubators and hatchers and in turn will reduce hatchability and produce low quality chicks [5].

The practices for keeping hatching eggs in a good sanitary condition require frequent collections and adequate cleaning and disinfection. Therefore, the eggs must be as quick as possible thoroughly disinfected after being laid, by adequate methods and compounds [17]. Eventually, bacteria penetrate the eggshell and infect the embryo, causing losses in hatchability, therefore an effective hatchery sanitation program is critical to achieve a high level of hatchability and ensure the production of high quality chicks [14].

Egg weight loss is an important factor for affecting the hatching success, in many domesticated birds, the amount of egg weight loss is around 12-14% of initial egg mass by piping time [19].

The changeable results of egg weight loss percentage due to egg treatment with disinfectants are reasonable since the disinfectants might affect the cuticle layers and shell porosity. This assumption is confirmed by [4] who reported that any alteration or removal of the cuticle by sanitizers may have a significant impact on egg weight loss and hatchability. Also, [9] reported that too-fast moisture loss was disadvantageous for the normal embryonic development. Yet, the literature on the possible deleterious effects of fumigation on the cuticle is limited [7]. Propolis is a sticky resin produced by worker honeybees from substances collected from plants, and it has strong antibacterial and antifungal properties. Also, Reference [2] confirmed that eggs sprayed with propolis had lower egg weight loss than those from other treatments.

Hatchability percentages had been improved for groups of propolis and fumigation but fumigation had harmful influence on human health, while propolis could be used safely as natural disinfectant. Therefore, using propolis 14% could be recommended as a good alternative egg disinfectant for realizing the best results of hatching success [18].

On day eighteen of incubation embryonic weight for eggs treated by propolis 14% was the heaviest and the lowest one was recorded for control untreated group. The increase of embryonic weight for propolis disinfection is a good criterion for embryonic development and it supposed that this natural

disinfectant did not adversely affect the cuticle and eggshell properties [18]. The cuticle may be affected by application of sanitizers and subsequently altered embryonic development. Moreover, the decrease of water vapour loss in eggs treated by propolis may influence the development of the embryos [3].

Application of disinfectants and formaldehyde fumigation for eggs had significant influence on total bacterial count (TBC) in compared to untreated eggs either at day of laying or after four days of storage. The growth of bacteria will increase on the eggshell surface after storage for untreated eggs. Also, using propolis 14% and fumigation for eggs could be recommended for decreasing the TBC on egg shell surface. Most of the dipping disinfectants used had residual effect on egg shell surface for longer time as the most of the counts were decreased after four days of storage, while fumigation did not possess the same character of residually. Reduction of the bacterial count through application of disinfectants on eggshell surface such as propolis could be assumed a good method for diminishing the number of contaminated hatched chicks and in turn decreasing the cross contamination [18].

[1, 13] stated that propolis has antimicrobial against staphylococcus species. The application of ethyl alcohol 70% treatment on hatching eggshell significantly decreased TBC on the 4th day of storage time [18].

The hatchability of fertile eggs declined with long storage period and the most obvious effect observed in eggs stored for 14 days [8]. The disinfection process is complex and multifaceted, as well as influenced by a number of factor and conditions. Some of them are related to the properties of the used disinfection agent, others to the type and resistance of microorganisms or the environmental conditions in the area where the disinfection takes place [15, 11].

So, the aim of the present study was carried out to investigate the effect of time of hatching eggs disinfection by some egg disinfectants from different sources on egg and hatching characteristics

2. Materials and Methods

2.1. Breeder Flock and eggs

In total, 600 fertile and fresh hatching eggs (unwashed, feces-free) were obtained from broiler breeders (Saso; 35 wk of age) that were raised on a deep litter system in a private farm in Gharbiya Governorate, Egypt. The sex ratio was 1 male: 10 female and a photoperiod of 16L: 8D. The breeders were fed a breeder diet containing 2900 kcal of ME/kg and 20% CP. Water were provided adlibitum. Hatching eggs were collected in clean, sanitized plastic flats containers and selected as following; large and small size hatched eggs; misshapen, heavy dirty and cracked eggs were excluded. Eggs were collected between 6 h and 7 h a.m. Eggs were then transferred in special containers with room temperature at 18.5°C and relative humidity (RH) 75%. to the lab.

2.2. Preparation of Solutions

- A 14% propolis solution was prepared by mixing 860 ml of 70% ethyl alcohol and 140 g of propolis. Solutions were kept in a container, the top was sealed, and it was shaken well before use. Each solution

was filtered (coarse filter) separately and was kept in a clean, dark bottle at 4°C until use.

- A 0.5% TH4 solution was prepared by mixing 5 ml of TH4 and 995ml of sterile distilled water. Solutions were kept in a dark container.
- A 0.5% Virkon S solution was prepared by mixing 5 g of Virkon S and 995ml of sterile distilled water. Solutions were kept in a dark container.
- 70% ethyl alcohol was bought in a ready prepared bottle.

2.3. Experimental design and application of Solutions

The eggs were allocated randomly into three equal treatment groups ($n = 200$) according to the time of eggs sanitization from egg laying (before 6 hrs and after 6 hrs). The eggs in the first group were kept as a control without treatment. While, the eggs in the second group were disinfected before 6hrs from egg laying and subdivided into equal four subgroups treated by Propolis 14%, Ethyl alcohol 70%, TH4 0.5% and Virkon S 0.5 %, respectively. The eggs in the third group were disinfected after 6hrs from egg laying and subdivided into equal four subgroups treated by Propolis 14%, Ethyl alcohol 70%, TH4 0.5% and Virkon S 0.5 %, respectively. All eggs were stored for short period (7 days) at 15.5°C in a room with RH 75%. Because the propolis was dissolved in ethyl alcohol, ethyl alcohol (70%) was used as egg disinfectant in this study to determine whether there was any synergistic effect.

2.4. Measurements

2.4.1. Egg weight loss

All eggs were individually weighed to calculate egg weight loss during storage and incubation (Before incubation and on day 18 of incubation) was recorded.

2.4.2. Incubation and its Parameters

2.4.2.1. Incubation Management

Eggs were marked and weighed before incubated in a commercial private incubator (Kafr El-sheikh Governorate, Egypt) with a temperature of 37.5°C and 55% RH until day 18 of incubation then incubator conditions were changed to 37.2°C and 75% RH to work as a hatchery. Eggs were turned through 90° once every 2 hrs. The time of setting eggs in incubator was recorded for each trial to obtain the exact hatch time in hours and considered from zero hour setting. Eggs were candled at 7th day of incubation to detect the fertility and on day 14 of incubation to detect the livability.

2.4.2.2. Incubation period

Incubation period was calculated by counting the time elapsed from setting the eggs in the incubator till hatching of the last egg in the group.

2.4.2.3. Hatchability

Hatchability was expressed as percentages of hatched chicks from total fertile eggs set.

2.4.2.4. Chick hatching weight

After 21 days, chicks that had fully emerged from eggs were removed from incubation and each hatched chicks was weighed individually to the nearest 0.1 g and recorded as chick body weight at hatch.

2.4.2.5. Embryonic mortality

At 14 day, the eggs which show dead embryos on candling and unhatched eggs after day 21 of incubation were opened to establish the stage of embryonic mortality. The stages of embryonic mortality were classified as follows: day 8 to 14 early embryonic deaths (black-eye visible and embryo without feathers) and day 15 to 21 late embryonic deaths (full-grown embryo with feathers and embryo with yolk out or subtracted).

2.4.2.6. Chick Performance

All hatched chicks per each group were reared on a deep litter system (10 chicks/m^2) to determine their growth performance for 7 days. Chicks were weighed and identified with a leg ring number. During the 7 days of growing, a starter diet (3020 kcal of ME/kg and 23% CP) was provided adlibitum. Room temperature was set at 33°C. The photoperiod was continued light for 24hrs during the rearing period. At the end of 7 days, all chicks were individually weighed (without leg ring). For each chick, the body weight (BW) of day 1 (BW1) and the BW of day 7 (BW7) were used to calculate the growth performance (GP).

2.4.2.7. Total bacterial count (TBC)

5 eggs per each group at laying day before and after disinfection were taken for microbiological analysis and immediately placed in sterile plastic sac containing 40 ml of sterile disinfectant. A whole-egg washing technique was used to recover the shell-associated microorganisms for estimating the total bacterial count. Serial dilutions were made from all samples and then were inoculated into sterile Petri plates containing nutrient agar **[10] .** The plates were packed and incubated at 37^{o}C for 48 hrs and at the end of incubation, the plates were removed and colonies were counted and multiplied by the dilution factor. Colonies were measured as log cfu/egg.

2.5. Statistical Analysis

Data were tested for distribution normality and hemogensty of variance. It reported as means and analyzed by tow-way ANOVA using Graph Pad prism 5. Duncan post hoc multiple comparisons test evaluated the significance of difference among the different groups. The significance level was set at $P< 0.05$.

3. Results and Discussion

3.1. Egg weight loss

It has been demonstrated that if hatching eggs are not sanitized prior to incubation, excessive bacterial contamination and subsequent growth can lead to decreased hatchability, poor chick quality, growth and performance [16].

Egg weight loss is an important factor for affecting the hatching success. Data in table 1 revealed that, there was no significant difference in egg weight loss during egg storage period (7days) in relation to time of egg disinfectants (before and after 6 hrs from egg laying) and among different disinfectant used. On the other side, egg weight loss during incubation (from day 0 to day 18) in eggs disinfected after 6hrs from egg laying was the lowest in compared to those disinfected before 6hrs from egg laying. In addition, using propolis in hatching eggs disinfection reduced the egg weight loss in compared to other disinfectants used. These findings may be explained by the occlusion of egg pores due to the oily nature of propolis which diminished the evaporation of water vapour and egg weight loss percentage. Beside, disinfectants might affect the cuticle layers and shell porosity of treated eggs.

These results are in agreement with that recorded by [18] who said that all disinfection treatments had no significant influence on egg weight loss percentage throughout (6-10 and 11-18 days), also, treatment the eggs with propolis 14% decreased egg weight loss percentage in compared to other egg treatments.

Table 1: Effect of disinfection time on egg weight loss during storage and incubation of broiler breeder eggs treated by different disinfectants

Variable	Disinfection					Time		P- value		
	Virkon S	TH_4	Propolis	Ethyl alcohol	Control	Before 6 h	After 6 h	Disinf.	Time	Interact.
Initial Egg wt (g)	**64.50**	**64.09**	**64.34**	**64.19**	**64.66**	**64.50**	**64.62**	**0.595**	**0.508**	**0.556**
Egg wt after storage (g)	**59.71**	**60.23**	**60.54**	**60.40**	**60.81**	**60.60**	**60.88**	**0.451**	**0.504**	**0.526**
Egg wt loss after storage (g)	**4.79**	**3.86**	**3.80**	**3.79**	**3.85**	**3.90**	**3.74**	**0.544**	**0.706**	**0.930**
Egg wt at beginning of incubation (g)	**59.71**	**60.23**	**60.54**	**60.40**	**60.81**	**60.60**	**60.88**	**0.451**	**0.504**	**0.526**
Egg wt on the 18th day of incubation (g)	**52.89**	**52.18**	**56.58**	**55.45**	**50.85**	**52.87**	**54.97**	**0.105**	**0.089**	**0.711**
Egg wt loss after incubation (g)	**6.82**	**8.05**	**3.96**	**4.95**	**9.96**	**7.73^{a}**	**5.91^{b}**	**0.006**	**0.005**	**0.085**

*Means which superscript with different small letter (a,b,c,…..) differ significantly at ($P< 0.05$)

3.2. Embryonic mortality percentage

Total early and late embryonic deaths in eggs disinfected before 6hrs was lower than those recorded in eggs disinfected after 6hrs from egg laying. But, early and late embryonic deaths in eggs disinfected with TH4 were the highest in compared to other disinfectants used as shown in table 2. These results may be attributed to the increase of bacterial load on the surface of eggshell and bacterial multiplication either in the surface of the shell or inside the eggs or due to the toxicity of this disinfectant (TH4). The results of embryonic mortality are in accordance with those reported by [6] who mentioned that the microbes on egg shells of newly laid eggs can multiply rapidly when exposed to appropriate ambient conditions and penetrate the eggshell through pores, this could lead to dramatic reduction in hatching success. In addition, rapid egg sanitization killed the microbes on the eggshell surface before penetration through the egg shell pores and affected the functional properties of the eggshell with respect to egg water loss and gas exchange during incubation. These results complicate the situation regarding application of any new egg disinfectants, therefore eggshell permeability should be taken in our concept in choosing any method of egg disinfection.

3.3. Hatchability percent

An effective hatching eggs sanitization program is critical to achieve a high level of hatchability and ensure the production of high quality chicks. As, the hatchability percent was increased by applying the process of disinfection before 6hrs than when it done after 6hrs from egg laying (table 2). This result may be due to high bacterial load which penetrate the shell and infect the embryo, causing losses in hatchability beside, high egg weight loss. On the other hand, using propolis in egg disinfection lowered the hatchability percent in compared to other disinfectants used. These findings may be explained by propolis remains long time on eggshell and occludes the eggshell pores and subsequently prevents gas exchange.

Table 2: Effect of disinfection time on embryonic deaths and hatchability percentage of broiler eggs treated by different disinfectants.

Variable	Time	Virkon S	TH4	Propolis	Ethyl alcohol	Control
Early embryonic death before 14th day of incubation (%)	**Before 6 hrs**	**0**	**2**	**0**	**0**	**2**
	After 6 hrs	**2**	**2**	**4**	**2**	**2**
Late embryonic death after 14th day of incubation (%)	**Before 6 hrs**	**4**	**4**	**4**	**2**	**0**
	After 6 hrs	**8**	**10**	**6**	**4**	**4**
Total embryonic deaths (%)	**Before 6 hrs**	**4**	**6**	**4**	**2**	**2**
	After 6 hrs	**10**	**12**	**10**	**6**	**6**
Hatchability %	**Before 6 hrs**	**90**	**86**	**82**	**86**	**82**
	After 6 hrs	**86**	**82**	**80**	**86**	**82**

3.4. Hatching time

Range of hatch time is a good indicator for chick distribution in the hatchery and it is preferable to reduce this range and shorten the staying of chicks in the hatchery to avoid chick dehydration. Results in table (3) revealed that, there was no significant difference in hatching time for egg disinfected before and after 6 hrs from egg laying, as well as, using different disinfectants didn't affect hatching time. These findings are contrary to that recorded by [18] who mentioned that the shortest range between maximum and minimum hath time was recorded for propolis14%, while the longest range of hatch time was observed for control untreated group as well as, it was disagreed with those previously mentioned by [12] who reported that the shortest range of hatch time was recorded for chicks produced from eggs treated with natural disinfectants. This disagreement may be attributed to the climatic, breed and management differences between the two experimental environments.

3.5. Chick weight

Concerning, chick weight after hatching, time of eggs disinfection didn't affect chick's weight after hatching. But, using propolis in egg disinfection increased the hatching chick weight in compared to other disinfectants used (table 3). This finding may be due to the lowest egg weight loss recorded by using of propolis as an egg sanitization. But, there was no significant difference in chick weight after 7 days from hatching by application of disinfection before and after 6hrs from egg laying. On the other hand, the chick weight after 7 days from hatching was higher in chicks hatched from eggs disinfected with propolis than those hatched from eggs disinfected with other disinfectants. This may be attributed to large chick weight at hatching in eggs disinfected with propolis.

Table 3: Effect of disinfection time on hatch time and chick weight of broiler eggs treated by different disinfectants.

Variable	Disinfection					Time		P- value		
	Virkon S	TH_4	Propolis	Ethyl alcohol	Control	Before 6 h	After 6 h	Disinf.	Time	Interact.
Hatch time (hrs)	**488.25**	**487.75**	**488.5**	**487.75**	**488.5**	**488.15**	**487.90**	**0.109**	**0.099**	**0.733**
Hatching Chick wt (g)	**39.83**	**42.08**	**44.04**	**39.99**	**40.03**	**40.39**	**40.99**	**0.018**	**0.068**	**0.010**
Chick wt after 7 day (g)	**90.84**	**95.69**	**98.51**	**90.51**	**89.44**	**92.27**	**93.13**	**0.016**	**0.280**	**0.049**

3.6. Total bacterial count (TBC)

The ideal environment for the embryo development is the same needed for microorganism multiplication. Therefore, contaminated eggs will disseminate microorganisms in incubators and hatchers and in turn will reduce hatchability and produce low quality chicks [5].

In the present study, there was a correlation between time of disinfection and total bacterial count (TBC). As, applying the process of egg disinfection before 6hrs from egg laying lowered the TBC on eggshell surface in compared to application of it after 6hrs from egg laying as shown in table 4. Moreover, using the propolis in egg disinfection reduced the bacterial load on eggshell surface in compared to other disinfectants used. These results are in agreement with that recorded by [18] who said that when the concentrations of propolis increased from 7% to 14%, TBC decreased from 21.79 to 17.11 X 10^3 cfu /egg. These results indicate that reduction in the bacterial count by the application of disinfectants on eggshell surface such as propolis could be assumed a good method for diminishing the number of contaminated hatched chicks and in turn decreasing the cross contamination during incubation.

Table 4: Effect of disinfection time on TBC of broiler eggshells treated by different disinfectants (cfu per egg).

Variable	Disinfection					Time		P- value		
	Virkon S	TH 4	Propolis	Ethyl alcohol	Control	Before 6 h	After 6 h	Disinf.	Time	Interact.
Before Disinfection	**6.30**	**6.39**	**6.37**	**6.45**	**6.709**	**6.53**	**6.23**	**0.99**	**0.64**	**1.00**
After Disinfection	**4.37**	**4.21**	**2.36**	**3.45**	**6.709**	**4.40[a]**	**6.30[b]**	**0.009**	**0.005**	**0.019**

*Means which superscript with different small letter (a,b,c,…..) differ significantly at (P< 0.05)

4. Conclusion

From these results, it could be concluded that application of egg sanitization process immediately after egg laying was beneficial in lowering TBC. Concerning different disinfectants, using propolis in the disinfection process reduced the egg weight loss and hatchability percent but increased the hatching chick weight.

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