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Research Article

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# *Comparison of Eucalyptus Extract and Formaldehyde on hatchability and Survival rate of chicks in Disinfection of fertile Eggs*

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## ABSTRACT

To compare the effect of eucalyptus extract and formaldehyde gas on the hatchability, survival rate, and microbial load of embryos and biological biomarkers of embryos in the fertile eggs of broiler chicken, 120 fertile eggs were divided into four groups (each group contained 30 eggs). The studied groups included the first and second groups in which eucalyptus extract at concentrations of respectively 1.100 and 1.200 was used as a disinfectant, the third group in which formaldehyde gas was used as a disinfectant and the fourth group as a negative control in which the studied eggs were sprayed with distilled sterilized water without using any disinfectant. All eggs were kept in a standard incubation system and the hatching rate was determined after 21 days. The hatched embryos were cultured to identify infection with E. coli. The hatched chickens were monitored for one week, and survival rate and the amount of using grain, overweight, and food conversion ratios were studied in different groups. The results showed that the disinfection of fertile egg with eucalyptus extract at the concentration of 1.200 was similar to that of formaldehyde, while the eucalyptus extract at the concentration of 1.100 can increase the hatching rate and the growth indicators. Therefore, it seems that using eucalyptus extract in the appropriate conditions and concentrations can be an appropriate alternative for formaldehyde.

**Key words:** *eucalyptus extract, formaldehyde, hatchability, Escherichia coli.*

## INTRODUCTION

Eggs can be used as an agent for the transmission of some pathogens common between humans and poultry, including *E. coli*, various species of *Salmonella*, *Listeria*, and *Campylobacter*. Although attempts have been made to minimize the health actions of microbial load on eggshell surfaces, some pathogen microbes are sometimes transmitted through the skin or its contents. The bacteria on the egg surface are multiplied after penetration into it, causing its corruption and even before its general corruption; it can reduce its nutritional value [1, 2]. Seyed Nejad et al. (2014) showed that *E. coli* and *Pseudomonas* have the highest sensitivity to eucalyptus. These researchers reported MIC and MBC of 8 mg/cc for *E. coli* in eucalyptus extract [3]. Wells et al. (2010) examined the effect of UV and oxygenated water at different time and concentrations on bacteria of eggshell surface. The results showed that the combination of hydrogen peroxide 1.5% and UV radiation for 8 minutes reduced the population of bacteria in each egg surface by 3log CFU [4].

Fasenko et al. (2009) examined the effect of oxidized electrolyzed water spray on hatching eggs and concluded that oxidized electrolyzed water significantly reduced the microbial load of the shell of hatching eggs without

negative effect on chicken weight when hatching and significantly reduces the mortality of chickens in the first two weeks. Therefore, this substance can be used to disinfect hatching eggs [5]. In 2003, the effect of two disinfectants with commercial names of Bio sentry 904 and Biox H, and electrostatic sprays for the elimination of *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli*, and *Salmonella enteritis* at the egg surface was evaluated. The study revealed that although these two compounds affect the reduction of eggshell surface bacteria, their use along with electrostatic spray significantly reduces the egg surface bacteria [6]. Therefore, the compounds that can reduce surface infection of egg-shell at all stages of production, packaging, and supply can be useful in reducing the transmission of pathogenic organisms to chickens resulting from the fertile egg as well as diseases resulting from this food source and increasing the shelf life of the eggs. Therefore, the present study was conducted for the aim of evaluating the effectiveness of an herbal disinfectant (alcoholic extract of eucalyptus) with commercial conditions (using formaldehyde gas) in disinfecting the surface infection of eggs and the biological characteristics of fertile eggs, such as hatching rate, survival of hatched chickens, microbial culture of chickens, and the probable casualties.

## MATERIALS AND METHODS

In the present study, to evaluate the effectiveness and compare the effect of eucalyptus extract and formaldehyde gas on hatching rate and survival of hatched chickens, 120 fertile eggs prepared from Behjoojeh Zagros Company were tested. These 120 eggs in the laboratory were divided into four groups (30 eggs in each group) with three replicates. The studied groups included:

In the first and second groups, eucalyptus extract was used at the concentrations of 1.100 and 1.200 as a disinfectant, respectively. In the third group, formaldehyde gas was used as a disinfectant. In the fourth group, as a negative control, the eggs were sprayed with sterilized distilled water without using disinfectant. In the first and second groups, the disinfectants of eucalyptus extract were used with concentrations of 1.100 and 1.200, in which the mentioned disinfectants were sprayed at the volume of 0.9 ml on each egg. In the third group, 20 cm formalin 37% and 40 g of potassium permanganate per cubic meter of closed space were used for 2 minutes. All the fertile eggs were incubated for 18 days at 37.5 °C and 55% humidity with daily rotation at least 4 times and at 45° angle relative to the perpendicular line. In the last 3 days of incubation, the fertile eggs were horizontally placed in a hatcher tray and incubated at 36.5 °C and 70% humidity without rotation.

In the first and second groups, the disinfectants of eucalyptus extract were used with 1.100 and 1.008 concentrations, and 0.9ml of the disinfectant solution was sprayed on each egg. In the third group, 20 ml of formalin 37% and 40 g of potassium permanganate per cubic meter of closed space were used for 2 minutes. All egg sprouts were incubated for 18 days at 37.5 °C and 55% humidity with daily rotation at least 4 times a day and at 45° angle to the perpendicular line. In the last 3 days of incubation, fertile eggs were horizontally placed in a hatcher tray and incubated without rotation at 36.5 °C and 70% humidity. After 21 days of hatching, the chicks were removed from the incubator and stored at 33-33 °C with free access to water and grain. The hatched chickens in all groups used 8% sugar juice plus multivitamins in the first 48 hours. At this stage, the number of hatching chickens from the fertile eggs in each group was calculated. At the end of day 22, the fertile eggs were opened and cultured from the yolk sac or heart (depending on the size, time, and the wastes of the embryos). The chickens were cared for 7 days and the survival rate of the chickens in different groups was calculated and compared. The yolk sac and heart of the chickens that died within 7 days underwent bacteriological tests. At the end of day 7, the overweight and rate of consuming grain by chicken in different groups were investigated. Data were analyzed using Sigma State 2.0 software. One-way analysis of variance (ANOVA) was used to examine the difference between the two groups and if there was a statistically significant difference, it was evaluated by the Tukey method. The level of significant difference was considered $P < 0.05$ in the studied groups. To test the production of indole, the suspicious colony was removed from the MacConkey medium, cultivated in peptone and ether medium, and incubated for 24 hours at 37 °C. Then, a few drops of the coax reagent were added to it and the result was read. The bacteria can produce indole, and tryptophan creates a red or pink ring at the surface of the medium by adding the coax reagent. *Escherichia coli* bacteria can create a red ring at the surface of the culture.

To count the total *E. coli*, 25 g of the test sample (in the case of shortage of 2.5 g) was poured into a sterilized bag and 225 ml of peptone water was added to it (if 2.5 g sample was used, 22.5 ml of peptone water was used). Then, it was stirred for 3-5 minutes. Then, test tubes were coded with decimal dilution numbers of $10^{-1}$ to $10^{-7}$, and 9 ml of peptone water was added to each tube. Then, 1 ml of contents of tube No. 1 was added and a serial dilution was prepared. For each of the dilutions, we considered two sterile blank plates and we coded the tubes with the corresponding tubes. Then, 1 ml of any dilution was added to each plate, 25 ml of the melted MacConkey medium

was added to it, and the plates beside the flame were rotated to mix and homogenize contents of the plate. The plates were incubated for 24 hours at 37 °C. Then, we counted the positive lactose colonies and multiplied by the relevant dilution and reported in the form of the number of colonies per one gram of the sample. To confirm the positive lactose colonies on the MacConkey medium as *E. coli*, five suspected colonies were transferred to the EMB medium, stored for 24 hours at 37 °C, and examined the colonies by differential IMVIC tests. The colonies with pink positive lactose, producing green metal glaze, positive in terms of indole and EMB, negative in terms of VP, and citrate reduction were identified as *E. coli*.

## RESULTS

The number of colonies of E. coli per gram of eggshell in different groups

Comparing the results of counting *E. coli* per gram of disinfected eggsshells shows that the number of colonies in the formaldehyde gas and 1% eucalyptus is significantly lower than that of other groups (P <0.05). The number of colonies of *E. coli* in the 0.5% eucalyptus extract group was significantly less than that of the control and more than that of formaldehyde and 1% eucalyptus extract (Table 1).

**Table 1:** The number of colonies of *E. coli* per gram of eggshell ($x10^3$) in different groups.

| Groups | Control | Formaldehyde gas | 1% eucalyptus extract | 0.5% eucalyptus extract |
|---|---|---|---|---|
| The number of colonies of *E. coli* | $^{c}$78.0±37.2 | $^{a}$19.0±33.0 | $^{a}$26.0±64.0 | $^{b}$54.0±38.1 |

a, b, c: similar letters represent a significant difference among the groups (P <0.05).

### Rate of hatching and survival of chicken

**Table 2:** The rate of hatching and survival of chicken

| Groups | Control | Formaldehyde gas | 1% eucalyptus extract | 0.5% eucalyptus extract |
|---|---|---|---|---|
| Rate of hatching | $^{c}$16.79 | $^{a}$100 | $^{a}$83.95 | $^{b}$50.87 |
| Rate of survival of chickens | $^{b}$21.84 | $^{a}$100 | $^{a}$100 | $^{a}$23.95 |

a, b, c: similar letters represent a significant difference among the groups (P <0.05).

In the control, 1% eucalyptus, and 0.5% of eucalyptus groups, 19, 23, and 21 chickens out of 24 chickens were hatched, and in the formaldehyde group, all the chickens were hatched. Statistical analysis of the data shows that there is no statistical difference only between the formaldehyde group and the eucalyptus group A, and there is a difference between other groups. In this study, the formaldehyde group had maximum hatching and the control group had a minimum hatching, and this difference was significant (P <0.05). Statistical analysis of the data shows that using formaldehyde gas or 0.5% and 1% eucalyptus extracts can significantly increase chickens hatching and survival rates.

In the control group, 6 fertile eggs and in the groups with 1% eucalyptus and 0.5% eucalyptus, 2, and 4 fertile eggs were not hatched, respectively. *Escherichia coli* bacterium was isolated through the culture of the content of non-hatched eggs. Besides, until the end of the first week, three chickens in the control group and one chicken in the 0.5% eucalyptus group died. *Escherichia coli* was isolated from the yolk sac of all the chickens died during the first week.

### Growth indices

Comparison of growth indicators shows that using formaldehyde gas in the setter system significantly increased the weight of one-day and seven-day chickens, and the consumption of grain; while, it does not affect the food conversion ratio. There was no significant difference in the mentioned indicators among formaldehyde and 1% and 0.5% eucalyptus groups. Data analysis showed that all of the disinfected groups had significantly higher 1-day and 7-day weight, and grain consumption compared to the control group.

**Table 3:** Comparison of growth indicators in different groups.

| Groups | Control | Formaldehyde gas | 1% eucalyptus | 0.5% eucalyptus |
|---|---|---|---|---|
| One-day weight | [a]69.3±41 | [b]46.2±46 | [ab]91.1±44 | [ab]23.3±44 |
| 7-day weight | [a]63.6±2.133 | [b]22.7±145 | [ab]69.5±3.143 | [ab]63.6±8.140 |
| Food consumption in the first week | [a]36.8±110 | [b]39.4±7.118 | [ab]86.9±9.120 | [ab]27.5±115 |
| Food conversion rate until the end of the first week | [a]06.0±82.0 | [a]04.0±81.0 | [a]05.0±83.0 | [a]07.0±82.0 |

## DISCUSSION

Fecal contamination of egg may lead to the penetration of *E. coli* through the eggshell and may spread to the chickens during hatching. It is often related to the high mortality rates, or it may give rise to yolk sac infection [7]. 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 major mean of infection for the egg [8]. Before hatching, *E. coli* results in yolk sac infection and embryo mortality. The chicken can also be infected during or shortly after hatching. In these cases, retained infected yolk, pticemia, omphalitis, seand mortality of the young chicks up to three weeks of age are observed [8]. The 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 [7]. However, the contamination of eggshells is inevitable. Therefore, the disinfection of the fertile egg before setting in the hatchery is required to decrease egg contamination. The results of the recent studies show that disinfection of fertile egg yolks, in general, can reduce the infection of the embryos and increase the rate of hatching, reduce the mortality of the first week, increase weight, and increase food consumption in the first week; while, it does not have an effect on the food conversion rate at the end first week [9]. A comparison of the hatching rate in different groups shows that the lowest hatching rate belongs to the control group, that this difference is significant with other groups. The reason that the groups receiving the disinfectants could improve the hatching compared to the control group can be attributed to the growth of saprophytic bacteria on the eggshells. It is grown in the incubation system and causes the death of the embryo, while in all groups, the use of eucalyptus extract and formaldehyde gas has been able to minimize the number of bacteria in the shell surface. In the recent study, the number of *E. coli* colonies per gram of eggshell in the control group was 7 times more than that of the formaldehyde group and this difference can justify 20% of the difference in hatching rate. Several studies have been carried out on the antibacterial properties of eucalyptus, but the effect of this antimicrobial agent on the incubation device has not been evaluated so far. In vitro studies indicated that the main ingredient in eucalyptus extract is cineol and the major antimicrobial activity of eucalyptus is related to the presence of phenolic compounds and tannin in this plant. In various studies, antimicrobial activity against *E. coli* has been observed following the use of ethanol, methanol or hydroalcoholic extract. In most studies, it has been reported that eucalyptus extract has a better antimicrobial activity on the gram-negative bacteria, especially *E. coli*, compared to gram-positive bacteria.

Concerning the use of plant-based compounds for the disinfection of fertile eggs, there is only one study that examined the effect of essential oil of oregano in comparison with formaldehyde gas. Copur et al (2009) showed that essential oil of oregano significantly reduced embryo mortality and could be used as a natural disinfectant in the incubation system [10]. There is only one study about the effect of disinfectants on the survival rate of chickens and growth indicators, in which the effect of hydrogen peroxide in the form of fumigation on the rate of survival and growth indicators was investigated. It was shown that the use of hydrogen peroxide as a disinfectant of fertile eggs have not had any effect on the rate of survival of chicken, body weight, and food conversion ratio, but it increased the survival time of the yolk sac in the resulting chickens [11]. The results of this study are consistent with the recent study on the lack of effect of the food conversion ratio. However, concerning the effect of disinfection on body weight and food consumption, a recent study showed that disinfection with formaldehyde and eucalyptus extract can improve the body weight and food consumption in the first week, and this improvement may be due to reduced shell infection load and, consequently, a reduction in the infection of umbilical cord and yolk sac. The use of eucalyptus extract as an alternative to formaldehyde gas has not yet been studied. In the present study, it was shown that using eucalyptus at the dilution of 1 to 100 can compete with formaldehyde gas in the indicators measured in this study. However, due to the sensitivity and importance of disinfecting the fertile eggs in terms of survival rate and growth indicators until the end of the period and even the analysis of metabolic parameters such as susceptibility to ascites, and so on, more extensive studies are needed to be conducted. It has

been already indicated that the infection of the umbilical cord and yolk sac of the chickens in the first week of breeding affects the general indicators of growth and performance of the chickens.

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