

# The Role of Dipping Duck Hatching Eggs with Cherry Leaf Extract as Natural Sanitizers on Hatching Performance and Eggshell Bacterial Counts

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**Abstract.** The research aim is to study the role of dipping duck hatching eggs with cherry leaf extract as a natural sanitizer on hatching performance and eggshell bacterial counts. A number of 533 Alabio duck hatching eggs were used in this study. Hatching eggs produced from The College of Vocational Studies IPB University; were collected three times a day. Five hundred thirty-three hatching eggs were divided into five treatment groups. The first group was as control, the second was dipping into commercial disinfection, and the third and the fifth group were dipping into cherry leaf extract with concentrations 250 ppm, 500 ppm, and 750 ppm, respectively. The results showed eggshell bacterial counts from cherry leaf extract 750 ppm is not significantly different from commercial disinfection treatment. The bacterial count is lower than other treatments. The dipping treatment of 750 ppm cherry leaf extract in Alabio duck hatching eggs resulted in the lowest embryo mortality and the highest hatchability compared to other treatments. The highest hatchability percentage was in the cherry leaf extract treatment of 750 ppm (90.9%) and the lowest was in the control (75.2%).

## 1. Introduction

The development of poultry embryos requires an ideal environment, one of them is the cleanliness of the hatching eggs. Efforts to produce clean (physically and microbiologically) hatching eggs are carried out starting management on breeding farms. For example, providing nest boxes, collecting hatching eggs as often as possible (4-7 times per day), storing hatching eggs in a clean egg tray, and doing physical cleaning on dirty hatching eggs [1]. These actions do not guarantee the loss of microorganisms on the surface of the hatching eggshell or even those that have penetrated the interior of the egg. Hatch eggs contaminated with microorganisms cause a decrease in hatching performance such as increased embryo mortality and decreased hatchability. The correct egg sanitation process will play a role in reducing embryo mortality and correlate with hatchability. [2] stated that hatchability is directly related to embryonic mortality. An effective hatchery sanitation program is critical to achieving a high level of hatchability [3]. The fumigation process using formaldehyde gas has long been applied to the hatching egg sanitation process. This process plays a role in reducing and eliminating microbial contamination on the surface of the eggshell before the eggs are hatched using an incubator. However, formaldehyde gas which is made from the reaction of formalin and potassium permanganate (KMNO<sub>4</sub>) is a dangerous gas for both embryos and humans as operators of the hatching process. The dangers that formaldehyde can pose to humans include irritating, carcinogenic, and mutagenic.

Based on these conditions, it is necessary to research alternative materials for the process of sanitizing duck eggs as a substitute for the fumigation process of formaldehyde gas. Hatchery researchers report the use of natural sanitizing agents such as propolis [3], clove essential oil [2], garlic extract [4] gives a positive response to hatchability and has no negative impact on the development of poultry embryos. Cherry leaves (*Muntingia calabura*) contain several active compounds that can work as antibacterial. Flavonoids, saponins, and tannins contained in cherry leaves are antibacterial. This is in line with the research of [5], the results of the screening of phytochemical components in the ethanol extract of cherry leaves contained secondary metabolites: sterols, triterpenoids, flavonoids, alkaloids, saponins, glycosides, and tannins. Flavonoids and saponins have more concentrated intensity than other compounds. The results of the antibacterial activity test of 70% ethanol extract of cherry leaves showed antibacterial activity against *Bacillus subtilis* and *Shigella dysenteriae*, with a Minimum bactericidal concentration (MBC) against *Bacillus subtilis* of 6.25% and *Shigella dysenteriae* of 3.25% [6]. Cherry leaf ethanol extract is also known to have antibacterial activity against *E. Coli*, *P. aeruginosa*, *S. Typhimurium*, *S. aureus*, *B. subtilis*, and *C. Albicans* bacteria, and the highest inhibition zone diameter was shown in *S. aureus* [5].

Recent research has been carried out to use cherry leaves as an antiseptic in the animal husbandry sector, including as an antiseptic in the process of dipping the nipples of dairy cows [7] and cherry leaf extract as a sanitizing agent for hybrid duck eggshells [8]. The results of [8], ethanol extract of 20% cherry leaves showed significant results in suppressing embryo mortality and increasing hatchability in the hybrid duck egg hatching process. However, research on the application of a concentrated extract of cherry leaves as a sanitizing agent for Alabio duck hatching eggs and knowing its

role on hatching performance has not been carried out. The process of sanitizing eggshells using natural sanitation ingredients such as concentrated extract of cherry leaves is expected to be a solution for the negative impact of using formaldehyde gas on the fumigation process of hatching eggs. This research was conducted to examine the role of concentrated extract of cherry leaves as a natural sanitizing agent in the cleaning process of duck eggshells and its effect on the hatching performance of duck eggs.

## 2. Materials and Methods

This research was carried out at the poultry laboratory of Livestock Management and Technology Study Program, The College of Vocational Studies Bogor Agricultural University. Hatching eggs produced from Alabio duck were collected three times a day and stored no longer than 24 hours in the room with a temperature of 18°C. Five hundred and thirty-three hatching eggs were divided into 5 groups and replicated four times. Each group represented a sanitation treatment based on cherry leaf extract at various concentrations in comparison to a chemical disinfectant and a control. The research treatment is demonstrated in Table 1.

**Table 1.** Research treatment

Treatment	Concentration	Method
Water (38-40 °C)/Control (P1)	Water (38-40°C)	Dipping
Chemical disinfectant (P2)	0,15% (1.5 ml per 1 liter water)	Dipping
Cherry Leaf Concentrated Extract (P3)	250 ppm (50 ml Cherry Leaf Concentrated Extract 5 000 ppm, 950 ml Aquabides)	Dipping
Cherry Leaf Concentrated Extract (P4)	500 ppm (100 ml Cherry Leaf Concentrated Extract 5 000 ppm, 900 ml Aquabides)	Dipping
Cherry Leaf Concentrated Extract (P5)	750 ppm (150 ml Cherry Leaf Concentrated Extract 5 000 ppm, 850 ml Aquabides)	Dipping

Note: Extract per 1 liter water

### 2.1 Incubation

The hatching process uses an automatic incubator with a capacity of 1000 eggs per day on both the setter and hatcher machines. The machine was cleaned using detergent, disinfected, and fumigated. The temperature and humidity in the incubator were set to 37-38 °C and 60-70%, respectively. Eggs to be incubated are given the identity of the egg number on the shell. Eggs were turned on from the 3rd day of incubation until the 25th day. Turning eggs is done automatically every one hour with a tilt angle of 45°. The rest treatment for hatching eggs was carried out from the 17th to the 25th day, 2 times a day (morning and evening) for 15 minutes each. From the 26th day to the 28th day, the frequency of rest is increased to three times a day (morning, afternoon, and evening). Hatch eggs were candled on the 7th and 25th days to see the condition and development of the embryos during the hatching process. Observations on the seventh day of the incubation period were carried out to determine embryo development, the number of fertile and infertile eggs. In addition to distinguishing between live and dead embryos. Egg Infertile and dead eggs were removed from the setter machine. Candling on day 25 or at the time of egg transfer was done to see if the embryo was still alive or dead. The live embryos would be transferred to the hatcher (egg transfer process). The hatching process took place on the hatcher machine until the 28th day.

### 2.2 Embryonic Death and Hatchability

Eggs that failed to hatch were broken out and examined macroscopically. This observation was done to estimate the development of the embryo and determine the age of death. Embryonic mortality was recorded on the 7<sup>th</sup> day and 25<sup>th</sup> day during the hatching process. The embryo mortality was expressed as the percentage of fertile eggs. Hatchability was expressed as percentages of setting and fertile eggs.

### 2.3 Duck Weight at Pull Duck

Duck embryos began to hatch on day 28 and after the hatching process was complete, the ducklings are left in the hatcher machine until the feathers dry. Then the ducklings would be weighed and recorded as hatching weight. Afterward, the ducklings were removed from the hatcher to be transferred to the duck starter house.

## 2.4 Statistical Analysis

Statistical analysis was carried out using Minitab 17 software with the experimental design model as follows:

$$Y_{ij} = \mu + \tau_i + b_j + \epsilon_{ij}$$

Information:

$Y_{ij}$  : The observation record  
 $\mu$  : mean  
 $\tau_i$  : the effect of treatment  
 $b_j$  : the effect of group ke-j  
 $\epsilon_{ij}$  : the random error

Mean differences were separated by TUKEY HSD (honestly significant difference) test.

## 3 Results and discussion

The weight of hatching eggs of Alabio ducks ranges from 70.9 – 73.3 grams. The weight of Alabio duck eggs ranges from 59-65 grams/egg [9]. The duck egg weight which is proper for hatching is between 65-75 grams with a normal egg shape [10]. The eggs used in this study came from 1.5-year-old Alabio ducks. Alabio ducks reach sexual maturity at the age of 5-5.5 months with an egg production period of 2.5 to 3 years [9]. Based on this, the hatching eggs used to come from productive parent stock which is still in the standard age of rearing. Five sanitary treatments of duck hatching eggs and their effects on embryo mortality, hatchability, and hatching weight can be seen in Table 2

**Table 2.** Fertility, embryonic mortality, hatchability, and duck body weight

Treatment	Average egg weight (g)	Fertility (%)	Embryonic Mortality (%)	Hatchability (%)		Duck body weight (g)
				Egg set	Fertility	
P1	72,7	94,89	24,78	71,9	75,2	41,61
P2	70,9	95,92	18,20	78,6	81,8	42,85
P3	72,0	92,38	13,90	79,3	86,1	41,27
P4	71,0	92,29	11,14	82,0	88,9	40,93
P5	73,3	94,70	9,10	86,0	90,9	41,63

Note: P-value  $\geq 0,05$  ; not significantly different

The egg fertility rate shows a high percentage, ranging from 92.29-95.92%. This figure is following with previous research [11] that reported the fertility rate of Alabio duck eggs was 95.67%, and [12] which was 93.97%. The high fertility of duck eggs shows that maintenance management in breeding farms has been carried out optimally, including management of feed, mating, health, and also the age factor of the parent stock. The hatching eggs of the P1 group had the highest average embryo mortality of 23.4%, while the lowest embryonic mortality was in the P5 group. This is thought to be related to the level of cleanliness and the amount of microbial contamination in the hatching eggshells. The presence of microbes on the surface of the eggshell can penetrate the egg and can cause contamination of the embryo. This causes the death of the embryo in the hatching process. Based on [10], the dirty duck eggs will be easily contaminated with bacteria that enter through the pores of the shell and cause the death of the embryo. The microbes on eggshells of newly laid eggs can multiply rapidly when exposed to appropriate ambient conditions, and penetrate the eggshell through pores. This could lead to a dramatic reduction in hatching success [13]. The causes of high embryo mortality in the hatching process can be caused by bacterial and fungal contamination, poor egg storage conditions (storage room temperature), disease, genetics, damaged egg, and shell quality, and improper incubation conditions ([12]; [14]; [15]; 16]; [17]; [18]; [19]).

Previous research [20] reported the amount of TPC of duck egg shells sanitized with warm water was 4.19 log 10 CFU/egg, higher than sanitation using chemical disinfectants and concentrated extract of cherry leaf 750 ppm, 2.59 log 10 CFU/egg, and 2.97 logs 10 CFU/egg respectively. These results indicate that the eggshell sanitation process is not proper enough if it is only washed using warm water. Concentrated extracts of cherry leaves (250 ppm, 500 ppm, and 750 ppm) showed a lower embryonic mortality percentage than chemical disinfectants group treatment. These results are consistent with research results [8] that reported that dipping duck eggs using 20% cherry leaf infusion reduced embryo mortality by 43.42%. Cherry leaves (*Muntingia calabura*) contain several active compounds that can work as antibacterial. Flavonoids, saponins, and tannins contained in cherry leaves are antibacterial. This is in line with the research [5], the results of the screening of phytochemical components in the ethanol extract of cherry leaves contained secondary metabolites: sterols, triterpenoids, flavonoids, alkaloids, saponins, glycosides, and tannins. Flavonoids and saponins have more concentrated intensity than other compounds, while the result from [20] showed that the intensity of

tannins is more concentrated than that of alkaloids and flavonoids. The mechanism of antibacterial tannin is to inhibit extracellular enzymes of microbes, taking over the substrate required for microbial growth. Other mechanisms work directly on metabolism by inhibiting the process of oxidation, resulting in the release of water and gas in eggs can be prevented [21]. Cherry leaf ethanol extract is also known to have antibacterial activity against *E. Coli*, *P. aeruginosa*, *S. Typhimurium*, *S. aureus*, *B. subtilis*, and *C. albicans* bacteria, and the highest inhibition zone diameter was shown in *S. aureus* [5]. The bacteria *E. Coli*, *S. Typhimurium*, and *S. aureus* were the dominant bacteria in the eggshell.

Hatchability as a percentage of hatchability based on setting eggs and fertile eggs showed no significant difference in each treatment, however, the average percentage of egg hatchability in the P5 treatment group was higher than the other treatments. This is inversely proportional to the embryo mortality percentage. Low embryonic mortality percentage will lead to high hatchability. The dipping method of hatching eggs with cherry leaf extract of 250 ppm, 500 ppm, and 750 ppm showed a higher average egg hatchability than the treatment with warm water and chemical disinfectants. The use of natural sanitizing agents in the hatching egg disinfection process has been widely practiced, [3] reported the use of 14% propolis extract gave higher hatchability than the group of eggs fumigated with formaldehyde. On the other hand, [22] and [23] found that there were no significant differences between the hatchability of oregano oil and formaldehyde fumigation groups. [24] reported that the hatch of fertile eggs in alcohol and control groups have been slightly lower than oregano vulgaris and they found a significant difference between oregano vulgaris and formaldehyde fumigation in the hatchability of fertile eggs. Also, [25] stated that the highest significant percentages for hatchability of fertile eggs were recorded in egg groups with cumin 04%. On the other hand, formaldehyde fumigation recorded the worst and highest significant percentage of embryonic mortality. [26] reported the use of essential oils (EOs) for egg sanitizer have significant reductions in the number of total aerobic mesophilic bacteria (up to 80.77%) and fungi (up to 69.33%) on shells, with hatchability rates significantly improved by up to 12.59%.

Based on [8], the treatment of 20% cherry leaf extract was able to increase egg hatchability by 12.01% compared to the control group (without being sanitized). Factors that affect hatchability include quality and handling of hatching eggs, egg fertility, and hatchery management (temperature, the humidity of the setter and hatcher machine, egg turning). In this hatching process, the temperature of the setter and hatcher machines tends to be stable as well as humidity. The setter and hatcher machine's temperature is set at 37-38 °C and with relative humidity at 60-70%. The hatching weight of ducks in each treatment was not significantly different, with an average hatching weight of 40.93 - 42.85 grams per bird. The weight of day-old duck (DOD) in this study was the following [27], which is a minimum of 37 grams per bird. Hatching weight is strongly influenced by hatching egg weight and hatchery management.

## 4. Conclusion

The treatment of dipping 750 ppm cherry leaf extract on hatching eggs of Alabio ducks as a natural sanitation agent resulted in the lowest embryo mortality percentage with the highest hatchability percentage compared to other treatments. The use of cherry leaf extract also did not affect the hatching weight of the ducks produced. Based on the results, concentrated extract of cherry leaves can be an alternative to natural egg sanitizers.

## Reference

1. R.A. Ernst, F.A Bradley, U.K Abbott, R.M Craig, *Egg candling and breakout analysis*, ANR Publication 8134, California (2004). Available in <http://anrcatalog.ucdavis.edu/pdf/8134.pdf>.
2. G.D. S. Oliveira, V.M. dos Santos, S.T. Nascimento, J.C. Rodriguesy, Alternative sanitizers to paraformaldehyde for incubation of fertile eggs, *J. Poultry Science*, 1-6 (2019)
3. E.H.A Shahein, K. Sedeek Eman, Role of spraying hatching eggs with natural disinfectants on hatching characteristics and eggshell bacterial counts. *Egypt. Poult. Sci.* **34** (1), 211-228 (2014)
4. M. Baylan, G.C. Akpınar, S.D. Canogullari, T. Ayasan, The effects of using garlic extract for quail hatching egg disinfection on hatching results and performance, *Braz. J.Poult. Sci.*, **20**(2): 343-350 (2018)
5. W.P.C. Buihan, R.O. Rubio, Jr DLV, Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from *Muntingia calabura* L. leaves and stems, *Asian Pac. J. Trop Biome* **6**(8), 682–685 (2016)
6. A.D. Prasetyo, H. Sasongko, Aktivitas antibakteri ekstrak etanol 70% daun kersen (*Muntingia Calabura* L.) terhadap bakteri *Bacillus subtilis* dan *Shigella dysenteriae* sebagai materi pembelajaran biologi SMA kelas X untuk Mencapai Kd 3.4 pada Kurikulum 2013, *JUPEMASI-PBIO*, **1**(1) (2014)
7. D.R. Prasetyanti, C. Budiarti, D.W. Harjanti, Efektifitas daun kersen (*Muntingia calabura* L.) dalam menurunkan jumlah bakteri dalam susu dan peradangan pada ambing sapi perah, *J. Ilmu-Ilmu Peternakan*, **19**(1), 1-16 (2016)
8. F.H. Al Hakim, MN Huda, GD Fitri, D Ambarwati, H Tistiana, Pengaruh ekstrak daun kersen terhadap daya tetas dan mortalitas telur itik hibrida, *J. Ilmu-Ilmu Peternakan*, **26** (2), 8 – 13 (2016)
9. Direktorat Jendral PKH, Itik alabio (2021), Available in: <http://bibit.ditjenpkh.pertanian.go.id/content/itik-alabio>

10. A.R. Setioko, *Penetasan telur itik di Indonesia*. Wartazoa. **7**, 40- 46 (1998)
11. D. Darmawati, Rukmiasih, R. Afnan, Daya tetas telur itik cihateup dan alabio, *J. Ilmu Produksi dan Teknologi Hasil Peternakan*, **4**(1), 257-263 (2016)
12. D.S. Kumalawati, T. Susanti, *Effect of breed on fertility, hatchability and embryo mortality of Alabio, Mojosari and Peking x White Mojosari crossbred duck eggs*, in *Proceedings: Seminar Nasional Teknologi Peternakan dan Veteriner* (2019)
13. M.I. Cook, S. R. Beissinger, G.A. Toranzos, J.W. Arendt, Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. *J. Ecology Letters*. **8** (5), 532–537
14. E.E. Onbaşlılar, Ö. Poyraz, E. Erdem, Effects of egg storage period on hatching egg quality, hatchability, chick quality and relatif growth in Pekin ducks. *Arch Geflügelk.* **71**,187-191 (2007)
15. A. Abudabos, The effect of broiler breeder strain and parent flock age on hatchability and fertile hatchability. *Int.J. Poult Sci.* **9**,231-235 (2010)
16. C.W. van der Pol, I.A.M van Roovert-Reijrink, C.M. Maatjens H. van den Brand, R. Molenaar, Effect of relative humidity during incubation at a set eggshell temperature and brooding temperature post-hatch on embryonic mortality and chick quality. *Poult Sci.* **92**,2145-2155 (2013)
17. H. van den Brand, M.P. Sosef, A. Lourens, J. van Harn, Effects of floor eggs on hatchability and later life performance in broiler chickens. *Poult Sci.* **95**, 1025-1032 2016)
18. A. Addo, J.A. Hamidu, A.Y. Ansah, K. Adomako, Impact of egg storage duration and temperature on egg quality, fertility, hatchability and chick quality in Naked Neck Chickens. *Int J Poult Sci.* **17**,175-183 (2018)
19. A. Jabbar, A. Hameed, A. Yousaf, A. Riaz, Y.A. Ditta, The influence of hairline crack eggs on hatchery parameters and performance of chicks. *World's Vet J.* **9**,76-83 (2019)
20. G. Ayuningtyas, R. Martini, W. Yulianti, The potential Of cherry leaf extract (*Muntinga calabura* L) s a sanitary agent for eggshell in the alabio ducks hatching process, *J. Sains Terapan*, **10** (2), 50 - 61 (2020)
21. Y. Nurwantoro, B. Resmisari, Pengaruh perendaman jus daun sirih (*Piper Betle* LINN) terhadap jumlah bakteri pada telur itik, *J. Indonesia Tropic Animal Agriculture*, **3**, 156-160 (2004)
22. I. Yildirim, M. Ozcan, R. Yetisir, The use of oregano (*Origanum vulgare* L.) essential oil as alternative hatching egg disinfectant versus formaldehyde fumigation in quails (*Coturnix coturnix japonica*) eggs. *Food Control.* **15**,169-172 (2003)
23. G. Copur, M. Arslan, M. Duru, M. Baylan, S. Canogullari, E. Aksan, Use of oregano(*Origanum onites* L.) essential oil as hatching egg disinfectant. *African, J.of Bio.* **8**, 2531 -2538 (2010)
24. I. Yildirim, N. Ozcan, Use of oregano and cumin essential oils as disinfectants on hatching Quail eggs, *J.Anim.Res.Inst.* **11**, 61 -63 (2010)
25. Bekhet, M. Gamal, Impact of hatching egg disinfection on hatching characteristics and chick embryos, *Indian J. of Animal Research*, **55**, 353-358 (2021)
26. G.D.S. Oliveira, V.M. dos Santos, S.T. Nascimento, Essential oils as sanitisers for hatching eggs, *World's Poultry Science Journal* (2021).
27. Badan Standardisasi Nasional, Bibit niaga (final stock) itik Alabio meri umur sehari. SNI 7358:2008 (2008).