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The Effectiveness of Cherry Leaf Extract (*Muntingia calabura L*) as an Anti-Bacterial Against Hatchability of Kub Chicken Eggs in Artificial Hatchery

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ABSTRACT

The present study aimed to determine the effect of cherry leaf extract (*Muntingia calabura L*) on hatchability of Balitnak Superior Free Range Chickens (KUB) through artificial hatching. The doses of cherry leaf extract used in this study were 0%, 10%, 15% and 20%. The hatching eggs used came from KUB hens that were intensively reared in the poultry house at UPBS BPTP, North Sumatra. The procedures applied in this study were collection of hatching eggs, fumigation of machines and equipment, setting of machines and hatching eggs, egg washing with cherry leaf extract and hatching of eggs for 21 days. During the hatching process, observations were made on predetermined variables. The observed variables were egg shape index, egg weight loss, shell temperature and hatchability. The method used was completely randomized design (CRD) with 4 treatments and 100 replications. The results showed that the hatching egg shape index in this study was 75.77 – 77.05%. The lowest egg weight loss was at P0 (0%), while the largest weight loss was at P2 (15%). The highest hatchability was observed in P1 (10%) at 84%. The most effective P1 treatment resulted in the highest egg hatchability in the present study.

Keywords: Cherry leaf extract, Hatchability, KUB Chickens

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Introduction

Indonesia possesses a lot of potential for poultry genetic resources, one of which is the Balitnak Superior Free Range Chicken (KUB). KUB chickens are superior free-range chickens as a result of selection of native chickens from 6 generations by the Agricultural Research and Development Agency (Suryana, 2017). KUB chickens have many advantages, one of which is the ability to produce eggs in large numbers compared to ordinary free-range chickens. KUB chicken egg production reaches 160-180 eggs/year, and the peak egg production reaches 70% (Prabowo *et al.*, 2020). Meanwhile, the production of free-range chicken eggs in general is 132 eggs per head per year, incubating and not caring for their children is 115 eggs per head per year, and incubating and caring for their children until weaning is 52 eggs per head per year (Pramudyati, 2009). The demand for KUB chickens in all regions in Indonesia is currently very high. Thus, Balitnak Superior Free Range Chickens (KUB) chicken DOC is highly needed by

farmers in cultivation activities as laying eggs and producing meat. The superiority of KUB chicken, able to produce eggs in high numbers, can be directed to meet the needs of animal protein of Indonesia.

The population propagation of KUB chicken is currently experiencing problems due to limited DOC production. As a result, its distribution to all regions in Indonesia was not completed. The problem of multiplying the population of KUB chickens requires a solution to support the production of large number of DOC. One of the efforts to multiply the population of KUB chickens is through the artificial hatching method (hatching machine). The machine hatching method has the advantage of having a large capacity for hatching eggs, as well as setting the hatching period more effectively and efficiently. Machine hatching has been carried out a lot, but there are many reports that the hatchability produced by hatching using a hatching machine is still relatively low. This low hatchability is thought to be due to hygiene problems in hatching eggs or eggs that have been contaminated by Salmonella bacteria. Jones *et al.*

(2012) reported that *Staphylococcus aureus* and *Salmonella sp.* are bacteria that contaminate eggs, affecting hatchability. Both bacteria can cause hatching failure through the death of the embryo. Therefore, to achieve high hatchability, the disinfection process of KUB chicken eggs is crucial.

Cherry is a plant that functions as a shade and is often found in the tropics. The cherry leaves contain chemicals such as water, protein, fat, carbohydrates, fiber, ash, calcium, phosphorus, iron, carotene, tianin, ribofalin, niacin, tanins, saponins, flavonoids and vitamin C (Zakaria *et al.*, 2011). Kurniawan *et al.* (2013) reported that cherry leaves (*Muntingia calabura L.*) contain several active compounds that are able to work as antibacterial compound. Flavonoids, saponins and tannins contained in cherry leaves are antibacterial compounds (Puspitasari and Wulandari, 2017). Anti-bacterial compounds in cherry leaves can be used as ingredients to destroy *Salmonella* bacteria causing low hygiene in KUB chicken hatching eggs. Therefore, this study aimed to determine the benefits of cherry leaf extract (*Muntingia calabura L.*) with different doses and its effect on hatchability of KUB chickens.

Materials and Methods

Time and place of the research

This research was carried out from July to August 2020 at the Field Laboratory of the Chicken Source Seed Management Unit (UPBS) KUB Assesment Institute for Agricultural Technology (BPTP) North Sumatra.

Materials and tools

The tool used in this study was a fully automatic incubator with a capacity of 2,000 eggs (GIL-3500, US). The machine was equipped with a heater and a temperature control thermostat. The tools included digital scale with an accuracy of 0.02 g (AJ 3000, Osuka, Japan), infrared thermometer (UX-A-01, Germany), dry bulb and wet bulb thermometer (PD Tani Jaya, Indonesia), digital caliper 0-150 mm x 0.05 (Krisbow, Indonesia), and hygrometer (HTC-2, China). Other supporting tools used were egg trays, dippers, spray tubes for cooling eggs, digital cameras to document observations, and writing instruments to record data. The material used in this study was 400 fertile free range chicken eggs (KUB) obtained from the UPBS chicken coop of KUB Balitbangtan BPTP North Sumatra. The hatching eggs used were collected for a maximum of 7 days of storage referring to Alsoyabel *et al.* (2013). Cherry leaf extract with doses of 10%, 15% and 20% as well as aquades and ethanol were also used. Other materials used involved Potassium Permanganate (KMNO₄) and Formalin 40% for fumigation of hatching machines and other hatchery equipment.

Research procedures

The hatching procedure includes collection of hatching eggs, sanitation of incubating machines and equipment, selection and sanitation of hatching eggs with cherry leaf extract, setting of machines and hatching eggs, cooling of hatching eggs, and controlling the temperature and humidity of the incubator.

Collection of hatching eggs. The eggs used in this study were hatching eggs from the hen of Balitnak Free range (KUB) which were collected for a maximum of 7 days of storage (Alsoyabel *et al.*, 2013). The hatching eggs used in this study were obtained from KUB hens reared in intensive maintenance cages with uniform age in UPBS BPTP cages in North Sumatra with a male to female ratio of 1:10.

Fumigation of hatching machines and equipment. The hatching machine used was first fumigated using Potassium Permanganate (KMNO₄) and Formalin 40% with a ratio of 2:1. Other supporting equipment were also cleaned with disinfectant and then dried.

Setting machine and hatching eggs. The incubator used was a fully automatic incubator with a capacity of 2,000 eggs. The machine was equipped with an egg hatch rack that rotated automatically and set to rotate once every 3 hours on the 3rd day to the 18th day of hatching (setter). The machine was also equipped with ventilation and special cooling fans that were useful for air circulation of the machine to support the development of hatching egg embryos (Ipek and Sozcu, 2017).

During hatching, the temperature (°C) and humidity (Rh) of the incubator are set at 37.0-38.0°C, the engine humidity in the setter period is 60% and in the hatcher period close to 80% (Wilson, 1990). The eggs were then weighed, measured in length and width to determine the index of egg shape. Before being put into the incubator, the eggs were washed using a solution of cherry leaf extract according to the doses.

Washing hatching eggs with cherry leaf extract. Cherry leaf extraction was carried out at the Chemical Laboratory of Biological Materials, Faculty of Mathematics and Natural Sciences, University of North Sumatra (USU). The procedure for making cherry leaf extract starts from collecting cherry leaves in Medan Johor District, Medan City, then cleaning and drying the cherry leaves for 2 days. Afterward, the cherry leaves are mashed with a blender and weighed for 2,000 grams. Cherry leaf powder was macerated by soaking it in 70% ethanol solvent until it was submerged and while stirring. The maceration results were filtered using a glass funnel with cotton in it, the filtrate was concentrated using a rotary evaporator at a temperature of 600°C to produce a liquid extract, then evaporated in a water bath to produce a concentrated extract. Once the cherry leaf extract was ready, it was measured according to the treatments (P0, P1, P2 and P3) and diluted with distilled water. After washing with cherry leaf extract, the eggs were

then numbered to facilitate observation during hatching.

Egg hatching. The hatching eggs that have been washed with cherry leaf extract are then put into the incubator setter for 18 days and transferred to the hatcher machine until they hatch at the age of \pm 21 days. The temperature and humidity of the previously used incubator has been set according to the conditions for hatching chicken eggs. Observations were made at the hatching age of 0, 7, 14 and 21 days. During the hatching process, data were collected, namely, egg shape index, shell temperature, weight loss and egg hatchability.

Observed variables

Egg shape index. The egg shape index is the state of the egg shape calculated according to the reference from Narushim and Romanov (2002) with the following formula:

$$\text{Egg index} = \frac{\text{egg width}}{\text{egg length}} \times 100\%$$

Egg shape index was measured using a digital caliper with an accuracy of 0-150 mm x 0,05. Measurement of egg shape index was carried out before the eggs were put into the incubator (day 0 of hatching).

Egg weight loss. Egg weight loss is the percentage of egg weight lost from the 0th day of incubation (incubation) to the 21st day. The calculation of egg weight loss refers to Van der Pool (2013):

$$\text{egg weight loss (\%)} = \frac{\text{egg weight on day 0} - \text{egg weight on day 18 (g)}}{\text{egg weight on day 0 (g)}} \times 100 \%$$

Egg weight loss was measured using a digital scale with an accuracy of 0,05 g which was carried out on day 0 (before being put into the incubator), day 7, day 14 and day 21 of hatching.

Eggshell temperature

Eggshell temperatures are changes (increase or decrease) in eggshell temperatures during the hatching process. The eggshell temperature was observed on day 0 (before being put into the incubator), day 7, day 14 and day 21 of hatching. Temperature measurements were carried out using an infrared thermometer (Braun) (Ipek *et al.*, 2015).

Hatchability

Hatchability is the number of eggs that hatched successfully divided by the number of fertile eggs multiplied by 100% (El-hanoun and Mossad, 2008). The calculation of hatchability is carried out after the entire hatching process is completed.

Data analysis

This study used a completely randomized design (CRD) with 4 treatments and 100 replications. Each replication consisted of 1 hatching egg. The treatments applied in this study were:

P0 : Concentration 0% = without washing

P1 : Concentration 10% = 10 ml of cherry leaf extract + 90 ml of aquadest

P2 : Concentration 15% = 15 ml of cherry leaf extract + 85 ml of aquadest

P3 : Concentration 20% = 20 ml of cherry leaf extract + 80 ml of aquadest

The data was processed by analysis of variance (ANOVA) (Mattjik and Sumertajaya, 2013). Data analysis was carried out using SPSS version 22 (software). If the analysis of variance obtained significantly different results, then the study proceeded with the Duncan Multiple Range Test (DMRT) test to compare the mean between treatments. Data analysis is based on the equation (Steel and Torrie, 1991), namely:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Description:

Y_{ij} = observed response value,

μ = general average value

α_i = effect of treatment factor (P0, P1, P2, dan P3)

ϵ_{ij} = effect of treatment factor error on the j-th replication (1, 2, 3,...).

Results and Discussion

Egg shape index

The egg shape index is the ratio of the width and length of the egg and has an effect on egg hatchability (Lase *et al.*, 2021). Narushim and Romanov (2002) reported that eggs with normal physical index had an effect on embryonic development and hatchability. The shape index of hatched eggs in this study is presented in Table 1.

Based on Table 1, the length of KUB chicken eggs reached 49,0-49,4 mm. The average width of KUB chicken eggs treated with cherry extract was 37-38 mm. Similar to egg length, the cherry treatment on egg width also had no effect. In this study, the washing treatment method given to KUB chicken eggs did not make a difference in the egg shape index. This result is in accordance with the report by Sari *et al.* (2010), stating that the egg shape index has no effect on hatching. The egg shape index is influenced by genetic traits, race and the process of egg formation while in the parent's reproductive tract (Narushim and Romanov, 2002). The results showed that the index of KUB chicken eggs hatched in this study was 75.77-77.05%. The egg shape index of KUB chickens in this study tended to be the same as the normal egg shape index of free range chickens (76.01%) and Pelung chickens (76.72%) (Prilajuarti, 1990). Egg shape index is a characteristic that is unique to each species (Cucco *et al.*, 2012).

Egg shape index is influenced by genetic traits, race, and processes that occur during egg formation, especially when the egg passes through the magnum and isthmus (Dharma *et al.*, 2001). Ensminger (1992) explains that the causes of variations in egg shape between species or within avian strains are generally determined by pressure by the oviduct muscles, volume of albumen and the size of the isthmus, breed and variation of flocks, heredity, age at first egg laying,

Table 1. Shape index of hatched egg

Variable	Treatments (mm)			
	P0	P1	P2	P3
Length	49.3±0.40	49.1±45.05	49.4±0.29	49.0±0.30
Breadth	37.7±0.28	37.9±0.37	38.0±0.46	37.1±0.27
Index (%)	76.88±7.52	76.26±10.38	77.05±10.12	75.77±5.39

P0: Concentration 0%; P1: Concentration 10%; P2: Concentration 15%; P3: Concentration 20%.

egg-laying cycle and time to stop laying eggs. Yuwanta (2004) stated that the ideal egg has an egg shape index value between 70%-75%. It was further explained that the egg shape index value in chickens varied between 65% and 82%. The larger the egg shape index value, the more rounded the egg shape will be, and vice versa, the smaller the egg shape index value, the more oval the egg shape will be.

Egg weight loss

The results of observations on egg weight loss are shown in Table 2. On day 0, washing with cherry leaf extract had a significant effect on egg weight. The weight of the eggs used as control (P0) was 41.05 g which was smaller than the weight of the eggs treated with cherry leaf extract. Giving cherry leaf extract had a significant effect on egg weight in treatments P2 and P3, with 42.71 and 42.54 g, respectively. On day 7, the egg weight used as a control had an egg weight of 38.87 g which was smaller those of P1, P2 and P3 treatments, reaching about 40 g. The use of cherry leaf extract on the day 7 had a significant effect on egg weight.

On days 14 and 21, control egg weight (P0) was 36.97 g and 34.19 g lower than those of P1, P2 and P3 (on days 14; 37-38 g, on days 21; 35 g). On day 14, cherry leaf extract had a significant effect on egg weight. The results of hatching egg weights in this study did not significantly differ from that of a research by Iskandar and Sartika (2014) stating that the weight of the first egg of KUB chickens reached a weight of 35-36 g. The aforementioned weight will continue to increase to 45 g/egg at the end of the second month of production, so that the eggs are ready for hatching. The results of the research by Romjali *et al.* (2019) stated that the egg weight of KUB chickens at the age of 22 weeks (35% production) was about 38.56 g. Based on DMRT analysis, hatching age on days 0, 7, 14, and 21 revealed the smallest weight loss in treatment P0 (0 days 41.05; 7 days 38.87; 14 days 36.97; 21 days 34.19) and the largest in treatment P2 (0 days 42.71; 7 days 40.36; 14 days 38.75; 21 days 35.65). Of the four treatments (after further testing), the stable weight loss treatment was in

the second treatment (P1) with a washing dose of 10%.

Cherry leaf extract based on phytochemical test results showed that the extract contained alkaloids, flavonoids and tannins (Gilang *et al.*, 2020). Tannins have antibacterial activity. According to Nurwantoro and Resmisari (2004), the antibacterial mechanism of tannins includes inhibiting microbial extracellular enzymes, taking over the substrate needed for microbial growth, or working directly on metabolism by inhibiting the oxidation process, so that the release of water and gases in eggs can be prevented. Flavonoid compounds act as antioxidants and antiseptics, in some cases flavonoids can act directly as antibiotics by interfering with the function of microorganisms such as bacteria or viruses (Hermiati *et al.*, 2013).

Shell temperature

The results of observations on eggshell temperatures that hatched from several washing treatments with cherry leaf extract are presented in Table 3. Treatment hatching eggs experienced changes in shell temperature with increasing hatching age. The average shell temperature on the day 0 of hatching was 36.13°C, and on day 7 of hatching, it increased to 37.16°C. On day 14, the temperature increased to 38.19°C. The increase in shell temperature in this study was in accordance with a report from French (1997) according to which, the embryo temperature was lower than the machine temperature (in the early hatching period) because the embryo had not yet undergone metabolism. During the hatching process, metabolic heat production from the embryo raises the egg temperature above the incubator temperature.

On the 7th day of hatching, the lowest shell temperature was in treatment P3 (36.87) and the highest were in P0 (37.24), P1 (37.24) and P2 (37.28). On the 14th day of hatching, the lowest shell temperatures were in P0 (38.02) and P1 (37.89) and the highest temperature was in P2 (38.53). In the 21-day observation period, the lowest shell temperature was in P1 (37.07) and the highest in P0 (37.35) and P3 (37.36). On the 21st day of hatching, the eggshell temperature

Table 2. Weight loss egg

Treatments	Weight loss (gram)			
	0 day	7 th day	14 th day	21 th day
P0	41.05±4.14 ^b	38.87±3.62 ^b	36.97±4.08 ^b	34.19±4.03 ^b
P1	42.01±3.60 ^{ab}	40.02±3.30 ^{ab}	37.79±3.10 ^{ab}	35.31±3.35 ^{ab}
P2	42.71±3.96 ^a	40.36±3.67 ^a	38.75±3.75 ^a	35.65±3.97 ^a
P3	42.54±4.51 ^a	40.23±4.32 ^a	38.53±3.89 ^a	35.31±4.08 ^{ab}

^{a,b} Different letters on the same row indicates statistical difference (P<0.05) among treatments.

P0: Concentration 0%; P1: Concentration 10%; P2: Concentration 15%; P3: Concentration 20%.

Table 3. Temperature of hatched eggshells on washing cherry leaf extract

Treatments	Temperature (°C)			
	0 day	7 th day	14 th day	21 th day
P0	36.71±0.23 ^a	37.24±0.66 ^a	38.02±0.59 ^c	37.35±0.85 ^a
P1	36.39±0.12 ^b	37.24±0.37 ^a	37.89±0.51 ^c	37.07±0.66 ^b
P2	35.69±0.16 ^c	37.28±0.26 ^a	38.53±0.44 ^a	37.29±0.92 ^{ab}
P3	35.74±0.16 ^c	36.87±0.35 ^b	38.30±0.43 ^b	37.36±0.54 ^a
Average	36.13±0.17	37.16±0.41	38.19±0.49	37.27±0.74

^{a,b,c} Different letters on the same row indicates statistical difference ($P < 0.05$) among treatments.
P0: Concentration 0%; P1: Concentration 10%; P2: Concentration 15%; P3: Concentration 20%.

decreased to 37.27°C. The decrease in temperature occurred at the age of hatching on day 21 because it has entered the hatcher period or the egg hatching period. During the hatcher period, the engine temperature is lowered so that it also affects the hatching egg temperature. In this study, the increase in shell temperature was not affected by the washing treatment of hatching eggs. The increase in shell temperature was influenced by the metabolic activity of the embryo which increased with increasing hatching age. The heat produced by the embryo expelled through the eggshell is a biological response to complex interactions within the egg tissue (Youssef *et al.*, 2014). The increase in shell temperature in eggs is caused by metabolic activity of the developing egg embryo, so that this activity produces heat that is released in the form of evaporation through the egg shell (Sotherland *et al.*, 1987).

Hatchability

Hatchability is the most important parameter because it describes the number of eggs that hatch. It is the number of eggs that successfully hatched minus the total number of eggs or egg density during the study. The hatchability of KUB chicken eggs during the study is presented in Figure 1. The average hatchability in each treatment differed. The effect of using cherry leaf extract in washing KUB chicken hatching eggs in this study resulted in the highest hatchability in treatment P1 (84%). P1 with a concentration of 10% produced the highest hatchability compared to P0, P2 (15%), and P3 (20%). This result is presumably because cherry leaf extract has active substances that can inhibit the growth of contaminating bacteria. This result is in line with Sufian *et al.* (2013) who stated that the

active substance in cherry leaf extract is able to inhibit the growth of harmful bacteria in hatching eggs. The active substances that act as antibacterial compounds are tannins and flavonoids. Tannins as antibacterial substances play a role in inhibiting microbial extracellular enzymes, taking over substrates needed for microbial growth, or acting directly on metabolism by inhibiting the oxidation process, so that the release of water and gases in eggs can be prevented. Cherry leaf extract is able to act as an antibacterial compound as it has cytotoxic activity (Sufian *et al.*, 2013; Zakaria *et al.*, 2011).

Based on the results of this study, the use of 0-10% cherry extract is still quite good because it is above 50%. This result shows that the use of cherry extract at a dose of 10% can increase the hatchability of KUB chicken eggs, while P3 showed that the use of cherry extract 20% reduces hatchability. This difference is presumably because the use of cherry leaf extract at a dose of 20% causes the pores of the shell to close, thereby inhibiting the process of evaporation and removal of CO₂ gas from the eggs during hatching. The inhibition of evaporation from the egg causes the embryo to be unable to release heat and increases the occurrence of embryo death during the hatching process. This result is not in line with Affandi and Tang (2002) who stated that the use of cherry leaf extract as much as 10-20% can increase the hatchability of poultry eggs.

There are many reports on egg hatchability using machines resulting in low hatchability; one of the causes is thought to be the bacterial contamination of eggs which results in the undeveloped embryo of hatching eggs (Reijrink, 2008). Hatching eggs from broodstock and rearing

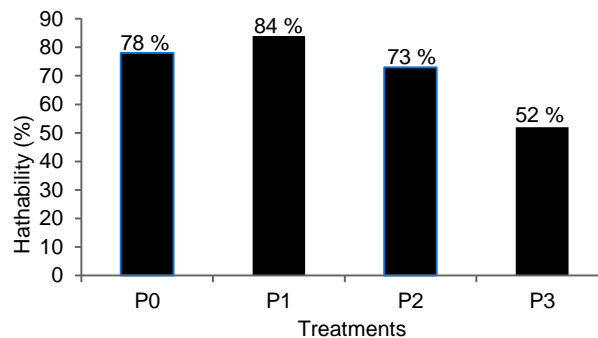


Figure 1. Egg hatchability chart (P0: Concentration 0%; P1: Concentration 10%; P2: Concentration 15%; P3: Concentration 20%).

cages have dirty shell and are potential sources of bacteria and fungi that can affect egg hatchability (Lase *et al.*, 2021; Harikrishnan *et al.*, 2013; Joseph, 2007). Koelkebeck (2010) reported that the hatchability of eggs is strongly influenced by the cleanliness of the eggshell. Lase *et al.* (2021) reported that microbiological contamination was strongly influenced by the ability of eggs to prevent microorganisms and bacteria from entering the egg through the pores of the eggshell.

Sulaiman *et al.* (2017) reported that cherry leaves can be used as antibacterial compounds because they contain tannins, flavonoids and saponins. Saponins contained in cherry leaves will interfere with the surface tension of the cell wall, due to this disturbance, antibacterial substances will enter easily into cells, and bacteria will die (Karlina *et al.*, 2013). Meanwhile, flavonoid compounds are polar compounds so they are easily soluble in polar solvents such as methanol, ethanol and acetone. According to Kurniawan *et al.* (2013), Flavonoids are disinfectant compounds that work by denaturing proteins which can cause the metabolic activity of bacterial cells to stop because all metabolic activities of bacterial cells are catalyzed by an enzyme which is a protein. A stop in bacterial cell activity will lead to the death of bacterial cells. Tannin compounds in cherry leaves can inhibit the activity of protease enzymes, inhibit enzymes in the transport of bacterial cell envelopes, destruction or inactivation of the function of genetic material. Also, tannins are able to shrink bacterial cell walls so that they can interfere with cell permeability. Due to disruption of cell permeability, these bacterial cells cannot carry out live activities so that their growth is inhibited (Mahardika *et al.*, 2014). The use of cherry leaf extract for egg washing in this study aims to inhibit the activity of bacteria that have the potential to affect the hatchability of eggs hatched by the machine. In this study, washing with a dose of 10% cherry extract had the highest hatchability.

Conclusions

The use of cherry leaf extract in KUB chicken egg dipping had a significant effect on egg shape index, egg weight loss, shell temperature and egg hatchability. washing with a dose of 10% cherry extract gave the highest hatchability.

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