



Effect of Kersen leaf extract disinfection of eggs on mortality, hatch weight, and quality of Mojosari ducklings

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Efeito da imersão em extrato de folhas de Kersen na mortalidade, peso ao nascer e qualidade dos patinhos Mojosari

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Abstract

This study aimed determine the effect of Kersen leaf extract (*Muntingia calabura* L.) on fertility, hatchability, embryo mortality, hatching weight, and quality of Mojosari ducks. A total of 200 hatching eggs were used, with a brood age of ± 48 weeks. The study was designed using a completely randomized design consisting of five treatments and four replicates, with each replicate containing 10 eggs weighing between 65–75 g. The treatments consisted of no dipping (P0, control), and 10 mL (P1), 15 mL (P2), 20 (P3) mL and 25 (P4) mL Kersen leaf extract + 100 mL distilled water. A distilled water-only treatment was

not included in this study. Data obtained were analyzed using analysis of variance and, if significant differences occurred, further tested with Duncan's multiple range test. The results showed that the administration of Kersen leaf extract did not have a significant effect ($p > 0.05$) on mortality, hatching weight, and quality of chicks. The average mortality ranged from 16.72 to 32.43%, hatching weight ranged from 38.95 to 40.02 g, and the Pasgar score value was in the range of 9.67 to 9.87. The best treatment was obtained in P1, which produced the lowest mortality rate (16.74%).

Keywords: Kersen leaf extract. Hatchery. Mortality.

Resumo

Este estudo teve como objetivo determinar o efeito do extrato de folhas de Kersen (*Muntingia calabura* L.) sobre a fertilidade, eclodibilidade, mortalidade embrionária, peso ao nascer e qualidade de patos da raça Mojosari. Foram utilizados 200 ovos férteis, com idade de cria de aproximadamente 48 semanas. O estudo foi conduzido em delineamento inteiramente casualizado, com cinco tratamentos e quatro repetições, sendo que cada repetição continha 10 ovos com peso entre 65 e 75 g. Os tratamentos consistiram em ausência de imersão (P0, controle) e adição de 10 ml (P1), 15 ml (P2), 20 ml (P3) e 25 ml (P4) de extrato de folhas de Kersen + 100 ml de água destilada. Um tratamento apenas com água destilada não foi incluído neste estudo. Os dados obtidos foram analisados por meio de análise de variância e, caso fossem encontradas diferenças significativas, submetidos ao teste de Duncan. Os resultados mostraram que a administração do extrato de folha de

Kersen não teve efeito significativo ($p > 0,05$) sobre a mortalidade, o peso ao nascer e a qualidade dos pintinhos. A mortalidade média variou de 16,72 a 32,43%, o peso ao nascer variou de 38,95 a 40,02 g e o escore de Pasgar ficou entre 9,67 e 9,87. O melhor tratamento foi obtido no grupo P1, que apresentou a menor taxa de mortalidade (16,74%).

Palavras-chave: Extrato de folhas de Kersen. Incubatório. Mortalidade.

Introduction

Mojosari ducks are known as one of the laying birds with high production levels. In their cultivation practices, farmers generally still apply a semi-intensive system that has the potential to cause contamination of microorganisms and feces in hatching eggs (Pangestu et al., 2021). Contamination of hatching eggs by dirt and microorganisms is a common issue in poultry production and can negatively affect egg quality and hatchability. Microbial contamination on the eggshell surface has been widely reported to increase the risk of embryo mortality due to the penetration of pathogens into the egg (Oliveira et al., 2022). According to Gunawan et al. (2022), the mortality rate of embryos in Mojosari duck hatching eggs that do not go through the sanitation process can reach 38.64%. Maintaining the cleanliness of hatching eggs is crucial, because the presence of dirt on the eggshell can trigger the growth of pathogenic microbes which will further inhibit embryo development (Alkhakim et al., 2016).

The hatching process is closely related to sanitation measures. The main objective of sanitization is to eliminate microorganisms on the eggshell surface that can potentially cause embryonic death and egg spoilage (Nadira et al., 2023). The types of disinfectants commonly used for disinfection include iodine, alcohol, quaternary ammonium compounds, formaldehyde, potassium permanganate, and phenol (Syamsi et al., 2018). However, inappropriate use of chemical disinfectants can be detrimental; for example, the use of formaldehyde in high doses can cause abnormalities in embryos. Some other chemicals are also known to be toxic, irritating, and have a strong odor. Therefore, alternatives in the form of natural

ingredients are starting to be considered as a solution to replace chemical disinfectants (Septiani et al., 2016).

The natural ingredients that contain flavonoids, saponins, tannins, phenolic compounds, and alkaloids function as antimicrobial, antibacterial, and antioxidant agents, so they have the potential to be used in the process of sanitizing hatching eggs (Mirza et al., 2017). One plant that has this potential is Kersen (*Muntingia calabura* L.) leaves. Compounds such as flavonoids, saponins, and tannins are known to be present in Kersen leaves (Vonna et al., 2021). This leaf extract is also proven to be able to inhibit the growth of various bacteria such as *Staphylococcus aureus* and *epidermidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Streptococcus viridans*, and *Escherichia coli* through the activity of its antimicrobial compounds, namely flavonoids, tannins, and saponins (Sadino et al., 2022).

Given the presence of bioactive compounds in Kersen leaves, the use of its extract as a natural disinfectant for hatching eggs is hypothesized to affect embryo mortality, hatching weight, and the quality of day-old ducklings. It is expected that egg immersion in appropriate concentrations of Kersen leaf extract may reduce embryo mortality and support better hatching performance and day-old ducks (DOD) quality compared to untreated eggs.

Material and methos

This research was carried out at Technical Implementation Unit for Livestock Breeding and Forage Crops, Kediri Regency, East Java, Indonesia. Research activities lasted from October 15 to November 15, 2024. All experimental procedures involving animals were reviewed and approved by the Animal Care and Use Committee (Ethical Clearance No. 88-KEP-FKHUB-2026), Faculty of Veterinary Medicine, Universitas Brawijaya. The study was conducted in accordance with the approved animal care guidelines.

The experiment was arranged in a completely randomized design with five treatments and four replications, so there were 20 experimental units. Each experimental unit consisted of ten eggs. The treatments consisted of no dipping (P0, control), and 10 mL (P1), 15 mL (P2), 20 (P3) mL and 25 (P4) mL Kersen leaf extract + 100 mL distilled water.

The extract concentration was expressed based on the volume of extract added because the extract mass concentration (mg/mL) was not determined in the present study.

Research material

The material used in this study was 200 hatching eggs. The hatching eggs came from Mojosari duck breeders with a sex ratio of 1:6 and a brood age of ±48 weeks. Eggs have been selected such as shell cleanliness, shape, and weight of hatching eggs. Semi-automatic hatching machine with a capacity of 300 eggs.

Preparation of Kersen leaf extract

The preparation of simplisia was carried out by adapting the method of Atam et al. (2020), namely by washing 1,000 grams of fresh Kersen leaves using water until clean, then drained and dried by aerating at room temperature 27-30 °C for five days. After the leaves are completely dry, rough cutting is done, then mashed using a blender until smooth. The results that have been smooth are then sieved to separate the fine from the rough, so that 300 g of simplisia powder is obtained.

Meanwhile, the process of making Kersen leaf extract follows the method of Giyatmi et al. (2024), namely by mixing 250 g of Kersen leaf simplisia into 2,500 ml of water, using a 1:10 ratio between simplisia and solvent. The extraction was carried out for 30 minutes with the temperature at the top of the pan reaching 90 °C, and during the process the solution was stirred periodically without being closed, so that some of the solvent could evaporate. After completion, the extracted solution was filtered while still hot using a filter cloth.

The phenolic compound composition of the extract was not quantitatively analyzed in this study. The extract was prepared using the decoction method. However, based on previous studies, Kersen leaves are known to contain bioactive compounds such as flavonoids, saponins, and tannins (Ratu et al., 2022). Therefore, qualitative phytochemical screening was conducted to confirm the presence of these compounds. The identification was based on characteristic color changes or foam formation after the addition of specific reagents (Table 1).

Table 1 - Qualitative phytochemical screening of Kersen leaves

Phytochemical test	Color change	Result
Flavonoid	Yellowish-red/orange	+
Tanin	Foam (1-10 cm)	+
Saponin	Greenish-purple color	+
Polifenol	Dark purple/black	+

Note: Positive results (+) indicate the presence of the corresponding compound. Source: Ratu et al. (2022).

Hatching process

The hatching process was carried out using one unit of a semi-automatic incubator. A total of 200 hatching eggs were distributed evenly into 20 experimental units, with each unit consisting of 10 eggs. The eggs from each treatment were randomly placed on different trays inside the incubator to minimize positional effects. All trays were arranged uniformly to ensure similar exposure to temperature and humidity conditions.

During incubation, the temperature was maintained between 37 and 38 °C and relative humidity at 60-70% from day one to day 25. In the last three days before hatching (day 18-21), the temperature was reduced to 36.5-37.5 °C, while humidity was increased to 70-80%. Eggs were automatically turned every hours from day 1 to day 18, after which turning was stopped to allow proper embryo positioning for hatching. The ventilation openings were adjusted gradually, starting at one-third open on day 3 and fully opened on day 7. Candling was performed on day 7 and day 14 to monitor embryo development.

Hatching occurred on day 21 of incubation. Newly hatched chicks were allowed to dry inside the incubator for several hours before being removed. After drying, the chicks were collected, counted, and evaluated for quality based on physical appearance and activity. Unhatched eggs were recorded to determine hatchability and mortality. All hatchlings were then weighed and assessed according to the experimental parameters.

Prior to incubation, all equipment, including water containers and egg racks, was washed with detergent and disinfected using a distan solution (15 mL per 10 L of water), then air-dried (Ramadhan and Afnan, 2019).

The interior of the hatching machine was thoroughly cleaned to remove dust and dirt. After drying, the equipment was placed inside the incubator and disinfected by spraying formalin. The incubator was then closed and left for three hours to ensure effective disinfection (Gunawan et al., 2022).

The incubator was subsequently operated for 24 hours prior to egg setting to stabilize internal environmental conditions. The temperature was maintained at 37-38°C, while relative humidity was kept at 60-70% by placing water containers inside the machine (Usman et al., 2022). After stabilization, the eggs were placed into the incubator, and the hatching process was carried out under these controlled conditions.

Hatching egg preparation

Hatching egg selection is done by considering several criteria, namely the shell must be intact (no cracks or breaks), normal or oval egg shape, and weight ranging from 65 to 75 grams, with an average egg weight of 69.20 grams. The coefficient of variation for egg weight was 1.15%, indicating that the eggs used had a high level of uniformity. The eggs used in this study were between 1 and 3 days old. Before treatment, the eggs were wiped to ensure that the shell surface was completely clean.

Eggs that had met the selection criteria and were cleaned were then divided into the five treatment groups (P0 - P5). The dipping process was carried out for 30 seconds according to the method used by Gunawan et al. (2022). After immersion, the eggs were drained to dry.

Statistical analysis

Embryo mortality was calculated as the percentage of fertile eggs that failed to hatch during the incubation period. Hatching weight was measured using a digital scale immediately after hatching when the feathers were dry and expressed in grams. DOD quality was assessed using the pasgar scoring method based on physical characteristics such as activity, feather condition, eye brightness, navel closure, and absence of deformities. Data were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, mean comparisons were performed using Duncan's multiple range test. Statistical significance was declared at $p < 0.05$.

Results

Based on the observation results, all treatments showed different results, although they did not give a statistically significant effect ($p > 0.05$). The average fertility, hatchability, mortality, hatching weight, and DOD quality of Mojosari ducks using Kersen leaf extract as a sanitizing agent for Mojosari duck hatching eggs are presented in Table 2.

Table 2 - Average results of mortality, day-old ducks hatching weight (HW), and Pasgar score

Treatment	Mortality (%)	HW (g)	Pasgar
P0	29.40 ± 10.90	38.95 ± 0.75	9.76 ± 0.21
P1	16.70 ± 6.42	38.83 ± 0.35	9.87 ± 0.12
P2	21.52 ± 6.96	39.44 ± 0.64	9.82 ± 0.13
P3	31.25 ± 8.29	40.02 ± 0.59	9.69 ± 0.18
P4	32.43 ± 14.06	39.75 ± 1.34	9.67 ± 0.23
p-value	>0.05	> 0.05	> 0.05

Note: Values are expressed as mean ± standard deviation. P0 (control) = no disinfection. P1, P2, P3, P4 = 10, 15, 20 and 25 mL of Kersen leaf extract + 100 mL of distilled water, respectively.

The highest egg mortality rate was observed in treatment P4 at 32.43%, while the lowest was in P1 at 16.72%. However, statistical analysis showed no significant differences ($p > 0.05$) among treatments, indicating that the use of Kersen leaf extract at different concentrations did not significantly affect embryo mortality during the incubation process.

For DOD hatching weight, the highest value was obtained in P3, with 40.02 g, while the lowest was found in P1, with 38.83 g. The difference in weight between treatments was relatively small, only 1.19 g. All treatments produced hatching weights that were still above the minimum limit of 37 g, according to the Standard National Indonesia (2008).

Assessment of DOD quality based on Pasgar scores showed that treatment P1 had the highest score of 9.87, while the lowest score was found in P4 which was 9.67. Although there were differences in scores between treatments, the results of statistical analysis showed that the differences were not significant ($p > 0.05$), and all scores were still in the excellent category (score >9).

Discussion

The absence of observable effects in this study may be associated with the limited effectiveness of the bioactive compounds in Kersen leaf extract when applied through the immersion method. Although Kersen leaves are known to contain flavonoids, tannins, and other phenolic compounds with antimicrobial and antioxidant properties, their activity may not be optimal due to insufficient penetration through the eggshell pores. The cuticle layer of the eggshell can act as a barrier, reducing the interaction between the extract and microbial contaminants on the shell surface. In addition, the concentration and duration of immersion applied in this study may not have been adequate to achieve effective sanitization. This may indicate that the applied concentrations were not sufficient to exert a meaningful influence on hatching performance under the conditions of this study. This condition may explain the limited impact of the extract on embryo development and duckling quality.

The absence of significant differences among treatments suggests that the antimicrobial activity of Kersen leaf extract was not sufficiently effective under the conditions of this study, which may be associated with its concentration and immersion-based application method. Although Kersen leaf extract has been reported to exhibit antimicrobial activity against various pathogenic microorganisms (Buhian et al., 2016), these effects are often observed under controlled conditions and may not be fully achieved in practical applications. Kersen leaf extract has been reported to have antimicrobial activity against several pathogenic microorganisms, including *E. coli*, *P. aeruginosa*, *B. subtilis*, *Candida albicans*, *Salmonella typhimurium*, and *S. aureus* (Buhian et al., 2016).

Flavonoids in Kersen leaves interfere with bacterial metabolic processes by damaging cell wall proteins (Wati and Wadjdi, 2023). Saponins disrupt the surface tension of bacterial cells, facilitating the entry of antibacterial compounds (Karlina et al., 2013). Meanwhile, tannins inhibit protease enzymes and disrupt cell membrane function, thereby inhibiting bacterial growth (Mahardika et al., 2014). However, the limited effectiveness in this study may be related to restricted penetration of these compounds due to the eggshell cuticle, as well as the potential blockage of eggshell pores when high concentrations are

applied, thereby disrupting gas exchange and embryonic respiration (Gunawan et al., 2022). Furthermore, immersion in highly concentrated solutions may lead to embryo mortality due to insufficient oxygen supply (Halim et al., 2022). In addition, high levels of bioactive compounds, particularly tannins, may exert toxic effects on embryos (Mulyani and Johan, 2020). Coagulation of proteins on the eggshell cuticle may also interfere with CO₂ and O₂ exchange (Wati and Wadjdi, 2023). Therefore, optimizing extract concentration and application methods, such as reducing immersion time or applying fine spraying techniques, may improve antimicrobial effectiveness without compromising embryo development.

The uniformity of incubation conditions, including temperature and humidity, as well as the relatively homogeneous egg weight and parental age, represents a strength of this study, as it minimized environmental variation and allowed for a more accurate evaluation of treatment effects. The use of a single incubator ensured stable incubation conditions, which supported consistent embryo development and resulted in relatively uniform DOD quality and hatching weight and across treatments. This finding is in line with Neonnub et al. (2019), who reported that stable incubation temperature optimizes embryo growth and development. In addition, the relatively uniform age of the parent stock may have contributed to the homogeneity of egg quality, including egg weight and shell characteristics (Ahyodi et al., 2014). These controlled conditions indicate that the similarity in results among treatments was more likely influenced by the limited effectiveness of the applied treatments rather than variability in incubation or egg quality.

The quality of DOD observed in this study was generally categorized as good, as indicated by high Pasgar scores. The Pasgar score is used to evaluate duckling quality based on physical and behavioral criteria, with a maximum score of 10 and a minimum score of 5, where scores ranging from 8 to 10 indicate the highest quality (Boerjan, 2002). These results suggest that the ducklings produced were in a healthy and viable condition at hatch. The application of Kersen leaf extract as a sanitizing agent did not significantly influence DOD quality, indicating that the tested concentrations were not sufficient to enhance duckling performance. Previous studies have shown that the effectiveness of plant-based antimicrobial agents depends on concentration levels, where sub-

optimal concentrations may not produce measurable effects, while higher concentrations may improve antimicrobial activity (Buhian et al., 2016). Therefore, further optimization of concentration and application methods is required to enhance its effectiveness.

The absence of omphalitis and the generally good physical condition of DOD across treatments suggest that overall egg sanitation and incubation conditions were adequate, likely due to the controlled incubation environment and proper handling practices applied throughout the study. However, since no microbiological analysis was conducted, the specific antimicrobial effect of Kersen leaf extract on eggshell contamination could not be confirmed. This limitation makes it difficult to determine whether the lack of significant improvement in embryo mortality, hatching weight, and DOD quality was due to insufficient antimicrobial activity or methodological constraints.

Therefore, future research should include microbiological analyses before and after egg sanitation to verify antimicrobial efficacy, as well as chemical characterization of the extract, including phenolic compounds, flavonoids, and saponins. In addition, improvements in the experimental design are needed, such as the use of standardized extract concentrations (e.g., mg/mL), optimization of immersion duration, and comparison with alternative application methods (e.g., spraying). Evaluating different exposure times and varying incubation conditions may also provide a better understanding of the potential effects of Kersen leaf extract on embryonic development and hatchability.

Conclusion

The immersion of hatching eggs using natural sanitizing agents, such as Kersen leaf extract solution, did not show a significant effect on the evaluated variables, including mortality, hatching weight, and DOD quality of Mojosari ducks. Although no statistical differences were observed, the treatment with 10 mL of Kersen leaf extract resulted in the lowest mortality rate (16.74%) among treatments. Thus, the use of Kersen leaf extract as a sanitizing agent does not impair incubation performance and may be considered a viable natural alternative, although further studies are required to confirm its effects under different experimental conditions.

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Authors' contributions

SP was responsible for designing the research, collecting and analyzing data and drafting the manuscript. EN provided supervision, methodological guidance, and scientific review. EN, RADA and AHS contributed to manuscript writing and manuscript editing. AAH provided advice and input in the preparation of the manuscript. All authors contributed to the interpretation of the results and approved the final version of the article for publication.

Data availability statement

The research data are not publicly available.

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