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Effect of sex ratio and different egg storage duration on fertility, hatchability, and normality of Kedu chicken eggs

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ABSTRACT

This study aimed to determine the effect of the Kedu chicken sex ratio and egg storage duration on fertility, hatchability, and normality of day-old chicks (DOC). Using 288 eggs from 12 hens and 6 roosters, researchers tested three SR groups (SR1=1:3, SR2=1:4, SR3=1:5) and two SD groups (SD1=3 days, SD2=7 days). Data were analyzed using one-way classification and N-way classification with the Statistical Analysis System (SAS) software package Ver 6.12. The results showed that with 3-day storage, SR2 had the highest fertility (89.58%), followed by SR1 (77.08%) and SR3 (72.91%). For 7-day storage, fertility was similar across SR groups (72.91%, 72.91%, and 70.83% respectively). Hatchability peaked at 82.18% (SR3) for 3-day storage and 81.11% (SR2) for 7-day storage. DOC normality was highest in SR1 (97.62%) and SR3 (97.22%) with 3-day storage, and in SD1 (88.69%) with 7-day storage. Analysis of variance showed that storage duration and sex ratio had no significant effect (P>0.05) on fertility, hatchability, or DOC normality. The conclusion of this study is that the optimal sex ratio can be achieved using SR3 (1:5), and the most efficient storage period can be achieved using LP1 (3 days).

Introduction

Kedu chicken is a distinctive local Indonesian chicken that originates from the Kedu District, Temanggung Regency, Central Java Province. Kedu chickens have the physical characteristics of dark feathers, a compact body, and high adaptability and endurance (Alfauzi and Hidayah, 2020). As a dual-purpose breed that produces both eggs and meat, Kedu chickens have the potential for development and are an alternative source for fulfilling local chicken protein needs and supporting community economies (Akbar and Ismoyowati, 2023). But thus far, the maintenance of Kedu chickens is still limited to local farms so that hatching is not optimal. Even though good hatchability is closely related to successful mating, which results in fertile egg hatching (Al Abror *et al*., 2018). Quality hatching eggs come from a well-managed breeding chicken program with the correct sex ratio, because it affects the efficiency and effectiveness of male and female utilization. Besides that, the length of egg storage is also an important factor as it can affect the quality of hatching eggs. Farmers often place eggs in the hatcher without considering the length of the stored eggs (Al Abror *et al*., 2018). Eggs that are stored for too long can decompose organic substances. According to Susanti *et al*. (2015), this decomposition leads to weight loss of eggs, affecting hatch weight. Prolonged storage lowers both quality and hatchability, and eggs should be stored for no more than seven days (Herlina *et al*., 2016). The hatchability of fertile eggs declines when they are stored for extended periods. The sex ratio should also be considered when producing fertile eggs. Low egg fertility results in low hatchability. This problem is certainly undesirable for farmers because it can cause losses. Based on these issues, to obtain superior stock from hatching, research is needed on the effects of egg storage duration and Kedu chicken sex ratio on egg fertility, hatchability, and DOC normality.

The purpose of this study was to determine the effect of the Kedu chicken sex ratio and length of egg storage on egg fertility, hatchability, and DOC normality. The benefits of this research are that it can be used as a reference for farmers in developing Kedu chicken breeding businesses, helping them determine the most efficient male-to-female ratio, and standardize egg storage duration. The hypothesis of this research is that there are sex ratio and storage duration factors that affect egg fertility, egg hatchability, and the normality of day-old chicks (DOC).

Materials and methods

Study design and sampling

The research materials used 288 Kedu chicken eggs, collected from mating 12 female Kedu chickens and 6 male Kedu chickens for incubation. To ensure high-quality gametes, all chickens were selected from the same age cohort during their peak production stage (approximately 32-48 weeks), prior to the onset of molting or a significant decline in hen-day production (HDP). Furthermore, males and females of compatible body size were chosen to facilitate successful mating and mounting. The number of eggs that hatched is presented in Table 1. The treatments consisted of two research factors and six incubation periods, as replicates. The sex ratio factor was grouped into 3 categories: SR1 (1 male: 3 female), SR2 (1 male: 4 female), and SR3 (1 male: 5 female). The storage duration factor was grouped into 2: SD1 (3 days) and SD2 (7 days).

Data collection

Data were collected according to the observed parameters. The observed parameters were egg fertility, hatchability, and day-old chick (DOC) normalcy. Egg fertility data were obtained by calculating the percentage of fertilized eggs relative to the total number of eggs set for incubation. Hatchability data were obtained by calculating the percentage of eggs that hatched to the number of fertile eggs. Data on DOC normal-

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Table 1. Number of hatching eggs.

Sex Ratio	Storage Duration (days)	Number of Eggs --(eggs)--	Replication --(times)--	Total Hatching eggs --(eggs)--	Timeline Collecting --(eggs)--
SR1[1]	SD1[2]	8	6	48	3
	SD2[2]	8	6	48	7
SR2[1]	SD1[2]	8	6	48	3
	SD2[2]	8	6	48	7
SR3[1]	SD1[2]	8	6	48	3
	SD2[2]	8	6	48	7

[1]SR1: Sex ratio 1 male, 3 females; [1]SR2: Sex ratio 1 male, 4 females; [1]SR3: Sex ratio 1 male, 5 males; [2]SD1: Storage Duration 3 days; [2]SD2: Storage Duration 7 days

cy were obtained by calculating the percentage of normal DOC relative to the total number of hatched chicks.

Statistical analysis

Data on egg fertility, hatchability, and DOC normalcy were subjected to a two-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS Ver 6.12. The model included the fixed effects of sex ratio, storage duration, and the sex ratio × storage duration interaction.

Results

The average results of macroscopic examination of fresh semen from male Kedu chickens are presented in Table 2. The average results of microscopic of fresh semen from male Kedu chickens are presented in Table 3. The data recapitulation of the research parameters is presented in Table 4. The average egg fertility rate is shown in Fig 1. The average hatchability is presented in Fig 2. Stages Leading Up to Egg Hatching is shown in Fig 3. The average DOC normality is presented in Fig 4 and 5. Normal Day Old Chicks is presented in Fig 6. Abnormal Day Old Chicks is shown in Fig 7.

Table 2. Macroscopic examination of fresh semen of kedu chickens.

Male chickens	Macroscopic Evaluation of Fresh Semen				
	Volume (ml)	pH	Colour	Odor	Consistency
A	0.40±0.13	7.00±0.32	Cream	Typical	Thick
B	0.27±0.14	7.17±0.82	Cream	Typical	Thick
C	0.37±0.23	7.00±0.32	Cream	Typical	Thick
D	0.28±0.13	7.25±1.08	Cream	Typical	Thick
E	0.37±0.18	7.17±0.75	Cream	Typical	Thick
F	0.37±0.18	6.67±0.52	Cream	Typical	Thick

Table 3. Microscopic examination of fresh semen of kedu chickens.

Male Chicken	Macroscopic Evaluation of Fresh Semen			
	Motility (%)		Abnormality (%)	Concentration ($x10^9$ sperm cells/ml)
	Mass	Individual		
A	2	59.17±3.76	22.92±2.46	3.10±0.44
B	3	67.50±9.35	18.75±2.62	2.98±0.58
C	3	70.83±8.01	18.75±3.79	3.17±0.39
D	2	55.83±7.36	26.25±3.45	2.88±0.29
E	3	64.17±13.93	23.75±6.07	2.98±0.60
F	2	60.83±12.01	22.92±5.79	3.00±0.39

Table 4. Research parameter data recapitulation.

Sex Ratio	Fertility (%)	Hatchability (%)	DOC Normality (%)
SR1[1]	75	74.99	93.15
SR2[1]	81.25	80.93	89.03
SR3[1]	71.88	80.73	92.78
P-Value	1.34	0.98	0.35
SD1[2]	79.86	79.54	95.04
SD2[2]	72.22	78.22	88.27
P-Value	2.63	0.11	2.52

[1]SR1: Sex ratio 1 male, 3 females; [1]SR2: Sex ratio 1 male, 4 females; [1]SR3: Sex ratio 1 male, 5 males; [2]SD1: Storage Duration 3 days; [2]SD2: Storage Duration 7 days

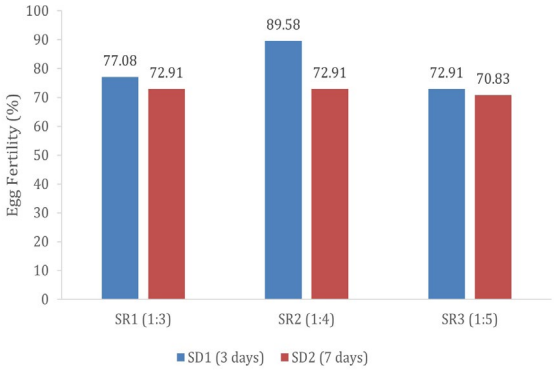

Fig. 1. Comparison of egg fertility across treatments.

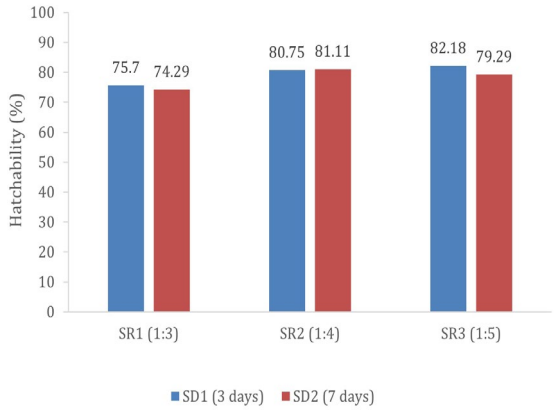

Fig. 2. Comparison of Hatchability Rates Between Treatments.

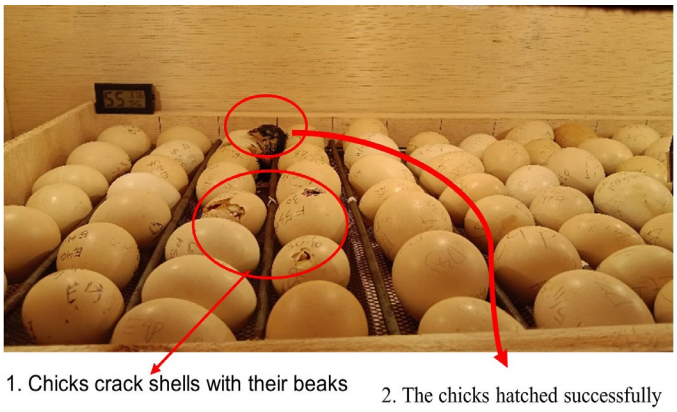

Fig. 3. Stages leading up to egg hatching.

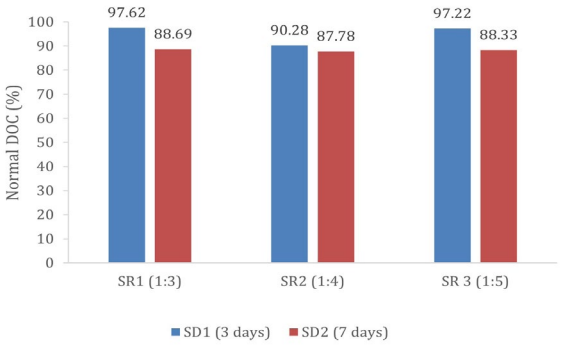

Fig. 4. Comparison of normal DOC across treatments.

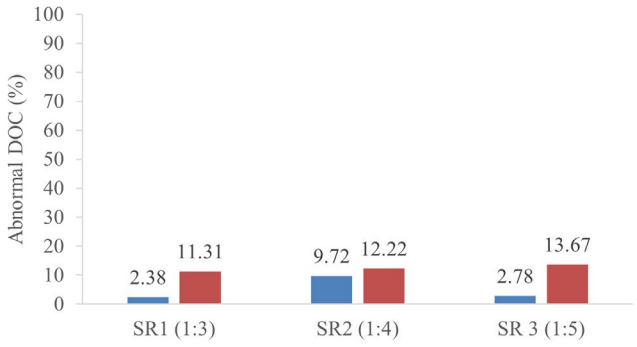

Fig. 5. Comparison of abnormal doc across treatments.

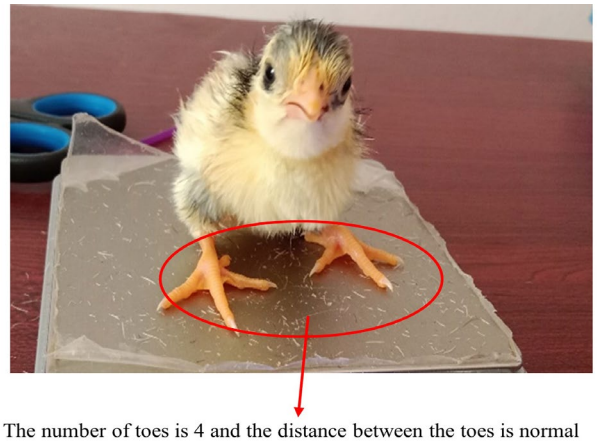

Fig. 6. Normal day old chicks.

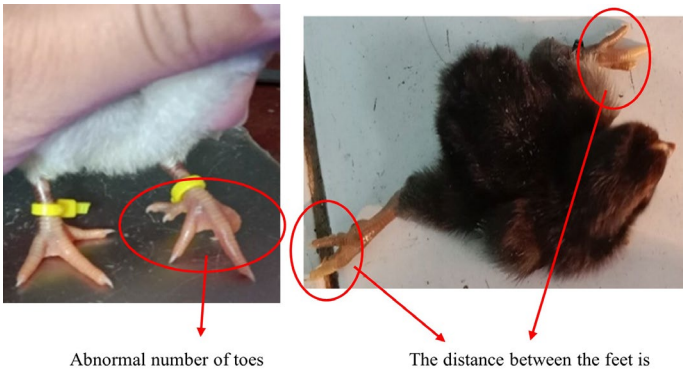

Fig. 7. Abnormal day old chicks.

Discussion

Table 2 showed that the volume of fresh semen from the six Kedu roosters ranged from 0.27 ml to 0.4 ml, which passes the examination because the standard semen volume for chickens is a minimum of 0.2–0.5 ml (Getachew, 2016). According to Table 2, the pH of fresh semen from each Kedu rooster varied between 6.67–7.25, which is higher than the results of Dako (2019), where the pH of local chicken semen ranged from 6.46 6.50. However, the pH of the semen obtained from each rooster was within the normal range. Wardhani *et al.* (2024) stated that the acidity level (pH) of local chicken semen is approximately 7 (normal pH). The pH value of semen should not be too high or too low because both excessive alkalinity and acidity can affect sperm viability or cause sperm death (Woli *et al.*, 2017). The average color of Kedu chicken semen is cream (Table 2.), indicating that it is relatively good. According to Zen *et al.* (2020), good-quality sperm has a white cream color. Cream colored semen, which is not translucent, is good and indicates high consistency. The smell of Kedu chicken semen was typical. According to Nugroho *et al.* (2016) who stated that the typical smell of normal local chicken semen is distinctive. Overall, the macroscopic quality of semen was considered to have passed the examination. Table 3 shows the mass motility results ranging from (+2) to (+3), which can be considered quite good according to Zen *et al.* (2020), who stated that good sperm quality has a mass motility of (+2) or (+3). The individual motility of semen from the six Kedu chickens ranged from 55.83% to 70.83%, which is considered less favorable because, according to Hafez and Hafez (2000), the individual motility of spermatozoa in normal poultry semen ranges from 60% to 80%. Based on Table 5, the average abnormality in the six males shows that only males B and C fall within the normal range, which is not greater than 20%. According to Zen *et al.* (2020), the percentage of spermatozoa abnormalities within the range of 5-20% per ejaculate can be used for AI, whereas if sperm abnormalities exceed 25% in one ejaculate, fertility can be reduced. The sperm concentration of the six Kedu chickens is considered quite good, as it falls within the range of 2.88–3.17x10^9 spermatozoa/ml (Table 3). According to Ervandi *et al.* (2020), good sperm concentration in chickens ranges from 2.5–3.5 x10^9 spermatozoa/ml, depending on the type of chicken, frequency of collection, and storage. In addition, concentration has a close relationship with consistency. Based on Table 2, the average sperm consistency is high; therefore, as shown in Table 3, it also has a good concentration. According to Zen *et al.* (2020), a thick sperm indicates a high concentration, whereas a thin sperm indicates a low concentration. Overall, the microscopic quality of semen can be considered to have passed the examination, although some evaluation parameters had low averages, which may have affected egg fertility.

The study examined fertility variations in Kedu chicken eggs across different sex ratios (1:3, 1:4, 1:5) and storage durations (3 vs. 7 days). While fertility rates ranged from 70.83% to 89.58% (Fig. 1.), statistical analysis revealed no significant differences between treatments, indicating that neither sex ratio nor storage duration substantially affected fertilization potential. The consistent fertility across sex ratios suggests that mating

frequencies allowed equal breeding opportunities for roosters, maintaining similar sperm quality (Astomo *et al*., 2016). However, semen analysis revealed suboptimal sperm motility (Table 3) and elevated abnormality rates in 4 of 6 roosters (Table 5), likely explaining the moderate fertility levels observed. Only roosters B and C showed normal sperm morphology (<20% abnormalities), supporting Zen *et al*. (2020) finding that >25% abnormalities reduce fertility. Interestingly, the 1:4 ratio showed higher fertility (89.58%) than 1:3 (77.04%), suggesting that balanced male health can maintain fertility even with skewed ratios (Reddish *et al*., 2018). The 2-week interval between male rotation and egg collection may have influenced results, as sperm can remain viable in hens' Sperm Storage Tubules for 3-4 weeks (Sugiyono and Musawati, 2024). Storage duration showed marginal effects, with 3-day storage yielding slightly higher fertility than 7-day storage, potentially due to better-preserved eggshell integrity (Susanti *et al*., 2015). While statistical differences were insignificant, prolonged storage risks degrading internal egg quality (Nova *et al*., 2014), emphasizing the need for optimal storage conditions (Sihombing, 2014). Key factors affecting fertility included sperm quality and rooster nutrition - particularly vitamin E levels, as deficiencies can impair fertility (Al Abror *et al*., 2018). The study preliminarily recommends a 1:4 sex ratio and 3-day storage for optimal results, though the minimal treatment differences suggest Kedu chicken fertility is resilient to moderate variations in these parameters when basic sperm quality and handling standards are maintained. These findings provide practical guidance for poultry breeding programs while highlighting the importance of semen quality assessment and proper storage protocols.

This study examined hatchability rates of Kedu chicken eggs across different sex ratios (SR) and storage durations (SD), with results ranging from 74.29% to 82.18% (Fig 2.). Statistical analysis revealed no significant effect (P>0.05) of either SR (1:3, 1:4, 1:5) or SD (3 vs. 7 days) on hatchability. The non-significant effect on fertility likely precluded a significant effect on hatchability, as successful fertilization is an absolute prerequisite for an egg to hatch (Asfaw *et al*., 2022). The primary sperm-related contributions to hatchability include sufficient sperm quantity to achieve fertilization and adequate sperm quality to ensure viable embryonic development, reducing early mortality. The similar fertility rates across SR groups likely contributed to this consistency (Astomo *et al*., 2016), though a trend toward higher hatchability was observed with increasing female numbers (SR3=1:5). This may reflect improved social dynamics or egg quality, as group interactions and hen health can influence laying behavior and egg viability (Khatun *et al*., 2019). Egg quality factors including selection criteria (size, shell integrity, air cell) and breeder hen management were critical to hatchability (Syamsudin, 2016). Despite using the most skewed ratio (1:5), SR3 achieved the highest hatchability, underscoring the importance of optimal breeding stock and handling practices. Incubator management by personnel also played a role (Fig 3.), as improper techniques (e.g., careless egg turning) can reduce hatching success (Al Abror *et al*., 2018). Storage duration showed no significant impact, though 3-day storage yielded marginally higher hatchability than 7-day storage. While eggs can remain viable for up to 9 days under proper conditions (Herlina *et al*., 2016), extended storage risks pore enlargement, microbial contamination, and chalaza degradation-the latter being critical for yolk anchoring and embryo development (Hartono and Isman, 2010; Sumbayak *et al*., 2020). Embryo mortality, inversely related to hatchability, may rise with prolonged storage due to these physiological declines (Astomo *et al*., 2016). The study preliminarily recommends SR3 (1:5) for optimal hatchability and SD1 (3-day storage) for efficiency. However, the minimal differences between treatments suggest that Kedu chicken hatchability is resilient to moderate variations in SR and SD when baseline egg quality and handling protocols are maintained. These findings highlight the need for rigorous breeder selection and storage management to maximize poultry productivity.

This study examined factors influencing the normality of Day-Old Chicks (DOC) in Kedu chickens, with particular focus on sex ratio, incubation conditions, genetic factors, and egg storage duration. Normal DOC exhibited complete limbs, proper mobility, correct feather coloration, and absence of abdominal distension, while abnormal chicks showed physical defects including limb abnormalities, weakness, or swollen abdomens (Fig 6 and 7.). Contrary to the initial hypothesis, sex ratio (SR) did not exert a significant direct effect on DOC normality. While incubation management is the paramount factor for chick quality (Molenaar *et al*., 2010; Oznurlu *et al*., 2016; Hedlund *et al*., 2021), the potential indirect pathways linking SR and storage duration (SD) to normality warrant exploration. The non-significant result for SR may be due to the experimental design; however, one potential mechanism could involve hen stress. In pens with a narrower sex ratio (e.g., more males per female), intense mating frequency may induce physiological stress in hens, potentially elevating reactive oxygen species (ROS) levels. This oxidative stress could compromise internal egg quality and reduce the egg's resilience during the subsequent storage period, ultimately deteriorating hatchling quality. Furthermore, while not directly measured, SR could influence sperm capacitation—a process critical for fertilization success that may be affected by male mating frequency. Similarly, for storage duration, the marginally better results for 3-day storage (LP1) over 7-day storage (LP2) align with established findings that the chalaza's function diminishes over time, potentially leading to albumin-yolk imbalances (Halgrain *et al*., 2022) that jeopardize embryo development. The pull-chick process, consistent with Badaruddin *et al*. (2023), was standardized across all groups and thus did not confound the SR or SD comparisons. Ultimately, these findings suggest that for the Kedu breed under these conditions, the moderate variations in SR and SD tested were secondary to the strict maintenance of optimal incubation conditions and robust genetic stock in determining DOC normality.

In general, poultry farmers prioritize high hatchability over high fertility because hatchability reflects the success of producing healthy, viable day-old chicks (DOC), whereas fertility only indicates the percentage of fertilized eggs without guaranteeing that those eggs will hatch successfully. According to Campbell *et al*. (2017), although a high fertility rate is important, high hatchability directly determines production efficiency and the farmer's economic gain, since only the eggs that hatch yield chicks that are worth raising. Therefore, it can be concluded that farmers may refer to the highest hatchability data by using the optimal sex ratio SR3 (1 male : 5 females) and the most efficient storage period LP1 (3 days).

Conclusion

The conclusion of this study is that the optimal sex ratio can be achieved using SR3 (1 male: 5 female), and the most efficient storage period can be achieved using LP1 (3 days). The recommendation from the authors is that farmers should use a sex ratio of 1:5 because it results in the highest hatchability and stores eggs for up to three days for optimal and efficient results. It is also suggested that future researchers use a time interval before collecting the eggs of more than 3-4 weeks so that the sperm fertilizing the females does not originate from the SST.

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Conflict of interest

The authors have no conflict of interest to declare.

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