Effects of weight and storage duration of hatching eggs of Indonesian Local Chicken on several measures of internal quality and hatchability

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Abstract. Hatchability and some internal qualities of hatching eggs of Indonesian Local Chicken (ILC) were investigated from different egg weights and storage durations. A total of 950 eggs (including 120 eggs for internal quality measurements) were collected and selected from a flock of INC aged 55 – 60 weeks reared in the University Farm, during 3-week production not consecutively. The research was arranged as a 6 x 4 factorial based on a randomized completely block design, using 6 levels of egg storage duration of 1, 2, 3, 4, 5 and 6 days, and 4 levels of egg weight (EW), 3 replication of unequal individual amount in each replication unit. Before storing, the eggs in each group were classified according to their weights: (1) ≥35 - <40; (2) ≥ 40 -< 45; (3) ≥ 45 - <50 and (4) ≥ 50 - < 55gr. All eggs were sterilized using alcohol 70%, numbered and stored at room temperature 26-27°C with relative humidity 60-70%. The results indicated that the proportion of egg weight loss of lighter egg at different storage duration was mostly significantly higher (P<0.05) than the heavier one. During storage, as the proportion of EW, albumen decreased, yolk and shell slightly increased. Based on correlation coefficient analysis, irrespective of storage duration and egg weight groups, the results indicated that egg weight losses were more closely associated (P<0.05) with alteration in albumen weight than other traits. Hatchability of fertile eggs (%) of all ranges of EW were significantly decreased with longer storage time, and the heavier decreased more significant than that of the lighter one, particularly after 3 d storage. Hatchability of EW more than 40 g showed better at 2 d storage duration compared to that of 1 d storage. Hatchability of EW up to 45 g was significantly better compared to those of heavier EW. In conclusion, hatchability of hatching fertile egg of ILC can be maintained at a level higher than 75% up to 3 d storage duration, and then its decrease was markedly for the weight of the eggs heavier than 50 g.

1. Introduction

Indonesian local chicken (ILC) usually called “ayam Kampung” is a common livestock reared traditionally by the small farmer in most agriculture areas of Indonesia. In the last decade, the existence of ILC is not just a traditional activity, but many have cultivated it commercially, even though the
efficiency level is still very low compared to exotic breed. Various efforts have been made to increase the productivity of local chickens, including the genetic breeding by crossing with an exotic breed, nutrition and management.

The research about hatchery activities in the context of breeding chickens has been widely carried out, and mostly based on the exotic chicken breeds. The research reported herein is arranged to reveal the effect of storage duration for hatching eggs of ILC and is associated with the weight of the hatching eggs themselves which related to the age of the hen. To minimize the effects of storage duration on hatchability and chick quality, commercial hatcheries commonly set their eggs after storing for 3 to 5 days. Some hatcheries of the grand-grandparent breeding farm set their eggs after 7 days of storage duration as economic consideration [1]. In general, there is an indication that an increase in storage duration resulted in decreasing hatchability [2-7], decreasing chick quality [3,6], decreasing post-hatched growth performance [3,6], and increasing post-hatched mortality [8]. An average of daily reducing hatchability up to 7 days storage duration by 0.2%, and there after this value increase by 0.5% daily [7].

As the producer of hatching egg, there are some factors of breeder hen affecting hatchability, includes strain, health, nutrition, age, weight and quality of the egg produced, storage duration, sanitation and season of the year [9-14]. Because of storage, the quality of the hatching egg is depreciated, and it is attributed to alteration in metabolic activity and embryonic development, which in turn to depress hatchability. Some hatchery factors may be attributed to hatchability, including egg handling, storage room and incubation condition, such as temperature, humidity, turning frequencies, ventilation, and egg position [14,15]. It is therefore that prolonged storage duration has detrimental effects on embryo growth, embryonic mortalities, hatchability, and in fact reducing chick quality at hatching [2,6,17]. Egg weight is increased with increasing age of breeder hen. Egg with different sizes or weights, may have different physical and chemical properties and the quality which possibly affecting hatchability and in fact chick quality at hatching. Chick weight at hatching is strongly related to egg weight. Heavier chicks may present higher body development and smaller yolk sacs due to higher development during incubation, or less developed bodies and larger yolk sacs, allowing them to survive longer before exogenous feed is provided [18].

The present study aimed to reveal the effects of storage duration and egg weight on the internal quality of hatching egg and the hatchability of the fertile eggs.

2. Materials and methods
The present experiment is to elucidate the effects of the storage duration and egg weight on the internal quality of hatching eggs and hatchability of eggs of Indonesian local chicken.

The research was arranged as a 6 x 4 factorial experiment based on a randomized completely block design of 6 levels of egg storage duration of 1, 2, 3, 4, 5 and 6 d, and 4 levels of egg weight (EW): (1) ≥35 - <40; (2) ≥ 40 - < 45; (3) ≥ 45 - < 50 and (4) ≥ 50 - < 55gr, with 3 not-consecutive weeks as replication. A total of 950 eggs produced by a flock of INC (aged 50-60 week old) rearing in the University farm during 3 not-consecutive weeks were daily selected, collected and used in the experiment. The amount of egg used in every treatment unit was un-equal (presented in table 1).

All selected eggs were clean up using alcohol 70%, weighed, numbered and stored in a room of 26-27°C and relative humidity 60-70%. A total of 120 selected eggs were used to measure the internal quality of the eggs, which were the weight of shell, yolk, albumen as well as the weight of the whole egg. The rest amount of the selected eggs were set in a semi-automatic incubator at 37.5 °C (99.5 °F) and 55% relative humidity. The eggs were turned every 6 h. The fertility and dead embryo were examined by candling at d 7 of incubation, the infertile eggs and the eggs containing dead embryo were removed. Data were analyzed using Statistical Package Systat for Window vs 13.
3. Results and discussion
The research results reported herein is about the effects of two factors, egg weight and storage duration, and interaction effects between the two factors. The results (Table 2) of the variance analysis showed that there was no significant interaction effect between egg weight and storage duration. It can be interpreted that the effects of egg weight were not affected by the effect of storage duration, and vice versa. The lost weight from smaller eggs appeared to be higher than that of the heavier one. As the proportion of fresh egg weight, albumen weights were significantly lower with longer storage duration up to 6 d (P<0.01), while yolk and shell weights were slightly decreased (P<0.05). However, these three parameters were not significantly different with different egg weights (P>0.05).

Prolonged storage of hatching egg has a detrimental effect on hatchability. The result indicated that longer storage duration of hatching egg resulted in decreasing the hatchability significantly, and the decrease was higher in heavier egg group: the group of more than 50 g > the group of 45-50 g > the group of 40-45 g > the group less than 40 g. Therefore, the group of 35-40 g can maintain their hatchability at a higher level for longer storage duration compared to the heavier groups. The hatchability of the egg group more than 50 g, reduced significantly to the level less than 70% just after 3 days of storage duration.

Results of previous researches showed that longer storage periods was to longer incubation period, impairing embryo development-abnormality, and viability, lower hatchability and chick quality and weight [3, 15,19,20], higher embryo mortality during storage [21,22]. These conditions resulted from alteration of internal egg quality – egg characteristic. Alburnum pH increases with storage time, and this effect is more significant in young breeder eggs [23], albumen height decreases with storage time and breeder age [23] and yolk sac membrane elasticity decreases with storage time [24]. Because these egg characteristics form the micro-environment surrounding the embryo, it is possible that the changes in the egg characteristics also affect cell death, embryo viability, or both. Therefore, alterations in embryonic development and in the egg characteristics, merit to be discussed further.

At oviposition, the albumen viscosity is maximal and decreased gradually after oviposition and during storage. Soon after oviposition, water from the egg starts to evaporate to the environment as the difference of water vapor pressures between inside and outside of the egg. Egg water is mostly contained in albumen, and water loss from albumen occurs in two-direction, to the environment, and to the yolk. The rate of water loss to the environment is influenced by the environmental temperature, humidity, storage duration, and egg size – weight. Initially, water evaporating through the pores of the shell comes from the egg-shell membrane, and this water loss to some extend is replaced by the water from the albumen [25-27]. As a result, therefore, there were some alterations in the viscosity and osmolarity of the albumen and yolk, and these alterations may have a contribution as a mechanism involve n albumen thining [28,29]. There are some factors affecting the viscosity of the albumen by directly or indirectly influencing pH, including storage duration, storage conditions and age of the breeder flock [17,30-32]. The loss of albumen viscosity is not linear with temperature but increases progressively with increasing

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**Table 1.** Produced/selected hatching eggs before incubation and used in the experiment.

| Replication group | Storage duration (d) | Total |
|-------------------|----------------------|-------|
|                   | 1  | 2  | 3  | 4  | 5  | 6  |     |
| 1                 | 49/40 | 36/30 | 58/50 | 45/40 | 60/55 | 54/50 | 302/265 |
| 2                 | 67/60 | 76/70 | 58/50 | 68/60 | 61/55 | 74/70 | 404/365 |
| 3                 | 58/50 | 47/40 | 67/60 | 59/50 | 68/60 | 66/60 | 365/320 |
| Total             | 174/150 | 159/140 | 183/160 | 172/150 | 189/170 | 194/180 | 1071/950 |
storage temperature. Attributed to the present research results (table 2), which indicated that when eggs are set in the incubator on the days of oviposition, hatchability lower compared to eggs stored for 2 - 3 days. Presumably, high albumen viscosity in the fresh egg impedes oxygen transport to the embryo.

**Table 2.** The effects of storage duration and egg weight on egg weight loss, proportions of albumen, yolk and shell of the egg

| Egg weight | 1 | 2 | 3 | 4 | 5 | 6 | P value |
|------------|---|---|---|---|---|---|---------|
| 35 - 40    | 0.85 ± 0.01<sup>a</sup> | 0.95 ± 0.05<sup>b</sup> | 1.40 ± 0.05<sup>c</sup> | 1.58 ± 0.21<sup>d</sup> | 1.60 ± 0.08<sup>e</sup> | 1.76 ± 0.11<sup>f</sup> | P<0.01 |
| 40 - 45    | 0.82 ± 0.03<sup>a</sup> | 0.85 ± 0.03<sup>b</sup> | 1.37 ± 0.01<sup>c</sup> | 1.41 ± 0.11<sup>d</sup> | 1.42 ± 0.04<sup>e</sup> | 1.42 ± 0.04<sup>f</sup> | P<0.01 |
| 45 - 50    | 0.81 ± 0.02<sup>a</sup> | 0.85 ± 0.03<sup>b</sup> | 1.34 ± 0.05<sup>c</sup> | 1.39 ± 0.06<sup>d</sup> | 1.41 ± 0.04<sup>e</sup> | 1.60 ± 0.13<sup>f</sup> | P<0.01 |
| >50        | 0.81 ± 0.01<sup>a</sup> | 0.86 ± 0.01<sup>b</sup> | 1.38 ± 0.05<sup>c</sup> | 1.37 ± 0.08<sup>d</sup> | 1.40 ± 0.09<sup>e</sup> | 1.43 ± 0.16<sup>f</sup> | P<0.01 |
| P value    | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 |         |

| Storage Duration (d) | 35 - 40 | 40 - 45 | 45 - 50 | >50 |
|----------------------|---------|---------|---------|-----|
| Egg Weight Lost (% fresh egg weight) | 30.95 ± 0.55<sup>a</sup> | 31.30 ± 0.55<sup>a</sup> | 31.55 ± 0.55<sup>a</sup> | 31.80 ± 0.55<sup>a</sup> |
| P value              | P<0.05  | P<0.05  | P<0.05  | P<0.05 |

| Yolk (%) | 35 - 40 | 40 - 45 | 45 - 50 | >50 |
|----------|---------|---------|---------|-----|
| Albumen (%) | 53.55 ± 0.54<sup>a</sup> | 53.04 ± 0.52<sup>a</sup> | 52.40 ± 0.66<sup>b</sup> | 51.73 ± 0.28<sup>b</sup> | 51.04 ± 0.83<sup>c</sup> | 50.74 ± 0.53<sup>c</sup> | P<0.01 |
| P value   | P>0.05  | P>0.05  | P>0.05  | P>0.05 |
| Shell (%) | 11.78 ± 0.24<sup>a</sup> | 12.01 ± 0.19<sup>a</sup> | 12.47 ± 0.26<sup>b</sup> | 13.22 ± 0.61<sup>c</sup> | 13.48 ± 0.45<sup>c</sup> | 13.56 ± 0.46<sup>c</sup> | P<0.05 |
| P value   | P>0.05  | P>0.05  | P>0.05  | P>0.05 |
| Hatchability (%) | 87.83 ± 1.50<sup>a</sup> | 85.42 ± 2.95<sup>a</sup> | 82.86 ± 4.04<sup>b</sup> | 80.00 ± 0.00<sup>c</sup> | 75.67 ± 6.12<sup>c</sup> | 73.36 ± 9.43<sup>c</sup> | P<0.01 |
| P value   | P<0.01  | P<0.01  | P<0.01  | P<0.01 |

a,b,c,d,e,f: Mean values in the same row with different superscript letter differ significantly (P<0.05; P<0.01)

After oviposition, the embryo is exposed to different environmental factors, in the nest and in the storage room, which may affect embryonic development and viability, which is primarily affected by environmental temperature. In addition to the rate of water loss, the condition of the storage room (26-27 °C and 60-70 % rH) in the present experiment may also affect negatively the embryonic development and viability at the cellular level. Commonly, hatching eggs are stored in the cooling room having a
temperature of 21°C below normal incubation temperature. The main reason is to stop or to minimize the embryonic development, and may also to prevent bacterial growth in the incubator. In chicken, embryonic development is arrested after laying and cooling the eggs down to storage room temperature of between 20-21°C [33] or 24°C to 27°C [34]. This range of temperature is known as “physiological zero” [35] in which without any change in the gross morphology of the embryos during storage up to 21 days compared to eggs stored at 7.2°C, 12.8°C, or 18.3°C. However, some changes were shown in the cellular activity of the embryos. When storage duration increased, the number of mitotic and necrotic indexes (dead cell) increased at all three temperatures, particularly in the 12.8°C and 18.3°C temperature treatments [35].

A research on turkey egg [36] shown the total number of embryonic cell at oviposition (0 days), and after 2, 4, and 14 days of storage at 18°C was 32000, 21500, 19000, and 21000 respectively. In the first 48 h of storing, total number of embryonic cell decreased by 32%, and the decrease may be resulted from apoptosis and necrosis. The proportion of apoptotic cells in chicken embryo just after oviposition is 3.1%, and it increase to 13% after 14 days of storage at 12°C [37].

4. Conclusion
The storage had negative effects on egg weight loss, embryonic development, embryonic mortalities, hatchability, and sellable chick. Environmental factors mainly associated with storage are temperature, humidity, and storage duration related to physiological zero should be considered to minimize the negative effects of storage.

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