Buletin Peternakan 47 (3): 152-158, August 2023

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 230/E/KPT/2022
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v47i3.84112

Effectiveness Performance of Sodium Hypochlorite (NaOCl) to Increase Hatchability of KUB Chicken Eggs in Artificial Hatchery

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Article history
Submitted: 29 April 2023
Accepted: 18 July 2023

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ABSTRACT

This study aims to determine the effect of 40°C warm water and sodium hypochlorite solution (NaOCl) on the hatchability of KUB chickens through artificial hatchery. The NaOCl concentrations used in this study were 0.25% and 0.5%. The hatching eggs used are the production of KUB hens which are kept intensively in the UPBS Poultry Departmen, Assessment Institute for Agricultural Technology (AIAT) of North Sumatra. The research procedures applied in this study were collection of hatching eggs, fumigation of machines and equipment, setting of machines and hatching eggs, washing of eggs with cherry leaf extract and hatching of eggs for 21 days. During the hatching process, observations were made on the variables that had been determined. The observed variables were egg shape index, egg weight loss, shell temperature and hatchability. The method used was a completely randomized design (CRD) with 3 treatments and 300 replications. The results showed that the shape index of the eggs that hatched in this study was 76.71 - 77.38%, the P2 treatment experienced the most stable weight loss compared to other treatments. The conclusion in this study P2 (0.25% NaOCl) effective in the highest egg hatchability.

Keywords: Artificial, Hatchability, Hatchery, Hypochlorite, KUB chicken, NaOCl

Introduction

Balitbangtan Superior Kampung Chicken or known by another name KUB chicken, is a new type of strain that has many advantages as a native chicken variety. This type of chicken is the result of genetic engineering innovation from the Research and Development Agency of the Ministry of Agriculture, Livestock Research Institute, Ciawi Bogor (Suryana, 2017). The KUB strain was obtained from crossing pure native chickens for six generations. The selection used to obtain KUB lines was to increase egg production by reducing the incubation of the parents. This type of chicken has many advantages including having a variety of coat colors as is the case with ordinary native chickens, body weight can be reached at the age of 70 days, first egg laying at the age of 20-22 weeks with a body weight of the first egg laying between 1.2-1.6 kg . In addition, KUB chickens are capable of producing 100-180 eggs/head/year with peak egg production reaching 65-70 percent (Hidayat *et al*., 2011; Prabowo *et al*., 2020).

The demand for chicken KUB continues to increase, because the nature of this KUB chicken is quite adaptive in various regional conditions in Indonesia so that people like to raise this chicken. In addition, the government's program through the Ministry of Agriculture in terms of spreading KUB chickens in various regions in Indonesia has resulted in a very high demand for this type of chicken. As a result of the high demand from all regions in Indonesia for KUB chickens, day old chickens (DOC) for KUB chickens are also urgently needed (Hasyim *et al*., 2021).

In order to meet the demand for KUB chicken DOC from breeders in all regions of Indonesia, efforts to accelerate the increase in DOC are needed, one of which is by artificial hatching (machine hatchery). Artificial hatchery is a practical, effective and efficient way to get DOC quickly and in large quantities. The advantage of other artificial hatchery methods is that they can be applied by breeders to the traditional level. Artificial hatchery (machine) is an effective way due to the capacity of hatching eggs in large numbers, as well as setting the hatching period more effectively and efficiently. But in practice, machine hatching which is often done gives low egg hatchability. There are many reports of artificial hatching with very low hatchability. The hatchability of KUB chickens is 26.46% (Norma, 2021). One of the main factors suspected of causing low hatchability in machine hatching is because the eggs have been contaminated interfere with embryo growth during the hatching process (Joseph, 2007).

Staphylococcus aureus and *Salmonella sp*. are bacteria that cause contamination of eggs, and these bacteria greatly affect embryonic growth during growth which results in embryos not being able to develop and die before successfully hatching (Jones *et al.*, 2012). To avoid low hatchability in the artificial hatching method due to bacterial infection of the hatching eggs, an attempt was made to wash the hatching eggs of KUB chickens using the washing method using Sodium Hypochlorite solution (Harikrishnan *et al*., 2013; David and Munadziroh, 2005).

Sodium hypochlorite is a class of disinfectants belonging to the oxygenated halogenated group which is active against all bacteria, fungi, viruses, spores and parasites (Martindale, 1982). The anti-bacterial properties possessed by sodium hypochlorite can be used as an ingredient to destroy Salmonella bacteria that cause embryonic death in developing eggs during hatching. Hypochlorite contains a disinfectant which acts to kill bacteria that contaminate eggs, and is able to thin the cuticle layer on the eggshell (Harikrishnan *et al*., 2013). Harikrishnan *et al.* (2013) reported that the use of a hypochlorite solution resulted in the highest hatchability of 63%, compared to washing eggs using water and glutaraldehyde solution. Therefore, this study aimed to determine the benefits of hypochlorite solution with different concentration as an anti-bacterial and its effect on the hatchability of KUB chicken eggs.

Materials and Methods

This research was carried out from August to October 2022 at the Field Laboratory of the Chicken Source Seed Management Unit (UPBS) KUB Assesment Institute for Agricultural Technology (AIAT) North Sumatra.

Materials and tools

The main tool used a fully automatic incubator with a capacity of 2,000 eggs (GIL-3500, US). This hatching machine has a heater and a temperature control thermostat on the machine. The incubator is equipped with supporting tools, namely; hygrometer (HTC-2, China), digital caliper 0-150 mm x 0.05 (Krisbow, Indonesia), dry bulb and wet bulb thermometers (PD Tani Jaya, Indonesia), infrared thermometer (UX-A-01, Germany) , as well as a 0.02 g precision digital balance (AJ 3000, Osuka, Japan).

Other supporting tools in this hatchery, are; egg trays, spray tubes for cooling eggs, digital cameras to document observations, and stationery to record data. The material in this study were fertile KUB chicken eggs as many as 1000 eggs obtained from the UPBS Chicken KUB Balitbangtan BPTP North Sumatra cage. The hatching eggs used as the object of observation in this study were collected for a maximum of 7 days of storage, referring to Alsoyabel *et al*. (2013). Hypochlorite solution with doses of 0.25 and 0.5% and distilled water, warm water 40°C. Other materials used are Potassium Permanganate (KMNO4) and 40% Formalin which are used as ingredients for fumigation of hatching machines and other supporting equipment.

Research procedure

The procedures of this research were, starting with collecting the hatching eggs of KUB chickens, sanitizing the hatching machines and other equipment used, selecting and sanitizing the hatching eggs with cherry leaf extract, setting the machine and hatching eggs, cooling the hatching eggs, and controlling the temperature and humidity of the machine, and hatch during hatching runs.

Collecting of hatching eggs. The object of this research was the hatching eggs of Kampung Unggul Balitnak (KUB). Eggs used for research were collected for a maximum of 7 days of storage or shelf life (Alsoyabel *et al*., 2013). The parents that produce these eggs were kept in intensive maintenance cages with uniform parent ages in the UPBS AIAT North Sumatra cages. The ratio of male and female parents that produced eggs in this study was 1: 8.

Fumigation of hatching machines. The hatching machine was fumigated using 40% formalin and KmnO4, ratio at 2:1 before being used. Other research supporting equipment were also disinfected to ensure that the equipment were not contaminated with bacteria.

Setting machine. The incubator used in this study has a hatching capacity of 2,000 eggs and is of digital automatic type for temperature regulation and egg racks. The egg rack rotates automatically every 3 hours starting from the 3rd day of hatching (setter) to the 18th day of hatching (hatcher). The machine has a cooling fan and is equipped with ventilation to support air circulation inside the machine so that the development of the embryo inside the egg can be optimal (Ipek and Sozcu, 2017). The temperature (°C) and humidity (Rh) in the incubator were set at a temperature range of 37.0- 38.0°C . During the setter period the humidity was set at 80% while in the hatcher period it is reduced by 60% (Wilson, 1990).

Setting eggs. The first step in hatching eggs was weighing the eggs, then measuring the length and width of the eggs to get the egg shape index number. Before being put into the incubator, eggs were first washed using a hypochlorite solution with different levels (concentrations) according to the treatment.

Washing hatching eggs. Hypochlorite solution as a treatment in this study was obtained from the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. The concentration of hypochlorite solution used for washing eggs in this study was 0.25%, 0.5% and with warm water 40°C. Total solution of each 1 Liter of each solution with a washing duration of 5 minute (Harikrishnan *et al*., 2013).

Egg hatching. KUB chicken hatching eggs that have been washed with hypochlorite solution were then put into the setter machine from 1 day to 18 days of hatching, then transferred to the hatcher machine until they hatch at ± 21 days of hatching. The incubator used has previously been regulated by temperature and humidity according to the conditions for hatching chicken eggs (Narushim and Romanov, 2002). Observations on the research object, namely KUB chicken hatching eggs, were carried out at the age of 0, 7, 14, and 21 days of hatching. During hatching, data was collected namely, egg index, shell temperature, egg weight loss and egg hatchability.

Observed variables

Egg shape index. The egg index will affects the development of the embryo and determines the hatchability of the egg. 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:

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

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

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

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

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 temperature is the change in eggshell temperature either in the form of a decrease or increase in temperature during the 21-day hatching process. The eggshell temperature was measured 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 (Hasyim *et al.,* 2021).

Data analysis

This study used a completely randomized design (CRD) with 3 treatments and 300 replications. Each repetition consisted of one hatching egg. The treatment applied in this study can be illustrated:

P1: Washing with warm water 40°C
P2: Washing with warm water 40°C + Concentration 0.25% hypochlorite
P3: Washing with warm water 40°C + Concentration 0.5% hypochlorite

The data was processed by analysis of variance (ANOVA) (Mattjik and Sumertajaya, 2002). 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 (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 index is the ratio between the length and width of the eggs (Narushim and Romanov, 2002; Lase *et al*., 2021). The egg index affects the development of the egg embryo and determines the hatchability of the egg (Narushim and Romanov, 2002). The shape of the egg is affected by the width or diameter of the uterus when it produces eggs. The wider the isthmus diameter, the resulting egg shape tends to be round, and if the diameter of the uterus is narrow, the resulting egg shape index tends to be oval (Hasyim *et al*., 2021). The shape index of eggs that hatched successfully in this study is presented in Table 1.

Table 1. Shape index of hatched egg

Variable	Treatments (mm)		
	P1	P2	P3
Length	52.3±0.28	52.5±0.27	52.3±0.21
Breadth	40.0±0.23	40.3±0.23	39.9±0.26
Index (%)	76.71±5.28[a]	76.93±5.53[a]	77.38±5.17[a]

[a,b] Different letters on the same row indicates statistical difference (P<0.05) among treatments.
P1: washing with warm water 40°C; P2: Washing with warm water 40°C + Concentration 0.25% hypochlorite; P3: Washing with warm water 40°C + Concentration 0.5% hypochlorite.

The results of the analysis of the egg shape index data in Table 1 can be seen that the egg length of KUB chickens is at the same average number, namely 52.3-52.5 mm, while the average egg width is 39.9-40.3 mm. The length and width of the eggs in the three treatments were at the same rate. The egg shape index is a variable that is not affected by the treatment in this study. This variable is fixed and measured from the start before the research treatment is carried out, so it can be interpreted that the egg-washing treatment method with sodium hypochlorite solution has no effect on the egg shape index.

The egg index is influenced by the breed, genetic traits, and the process of egg formation while in the main reproductive tract (Narushim and Romanov, 2002). The hatching index in this study was 76.71-77.38% and the three treatments were not significantly different. the results of the egg shape index in this study were normal, according to reports from Hasyim *et al*. (2021) that the normal egg shape index in KUB chickens is in the range of 75-77%. The shape index of eggs that hatched successfully in this study also had the same results as those reported by Prilajuarti (1990) that the egg index for native chickens was 76.01%, and for pelung chickens was 76.72%. The egg index is a characteristic or characteristic found in each species (Cucco *et al*., 2012).

The egg index value in chickens varies from 65% to 82%. The greater the egg shape index value, meaning that the egg shape is more rounded, and conversely the smaller the egg shape index value, the resulting shape is more oval. Yuwanta (2004) reported that the ideal hatching egg has an index value of between 70-75%. Dharma *et al.* (2021) reported that the index value is influenced by genetic traits, breed and processes that occur during egg formation, especially when it passes through the reproductive tract in the magnum and isthmus.

Egg weight loss

The observed egg weight loss parameters are shown in Table 2. Based on Table 2 it can be seen that on day 0 washing eggs with hypochlorite had a significant effect on egg weight. In this period the P3 treatment was lower (45.55) than P1 (46.35) and P2 (46.34). On the day 7th the egg weight used in P3 had a weight of 43.52 g lower than the P1 and P2 treatments, respectively 44.28 and 44.49. At the day 14th of hatching, the weight of P3 (41.54) was lower than P1 and P2, respectively 41.96 and 42.25 g. In the last period of hatching (day 21st) the egg weight loss in treatment P1 showed lower results compared to treatments P2 and P3. Overall, the use of hypochlorite in washing hatching eggs has a significant effect. The P2 treatment showed a higher stable weight loss rate than the other treatments in all observation periods.

Based on DMRT analysis, it can be seen that the most consistent washing treatment experienced the highest weight loss in each period, namely P2, respectively 46.34 g (0 day), 44.49 g (7 days), 42.25 g (14 days) and 40.02 g (21 days). This study is in line with reports from Hasyim *et al*. (2021) that KUB chicken eggs during machine hatching experienced a weight loss of 0.2-0.3% in each hatching period. Of the three treatments after further testing, the egg washing treatment showed that P1 and P2 were not significantly different on day 0 and day 7th, whereas on day 14th and day 21st, P1 and P3 were not significantly different. The weight loss that occurred in all treatments in this study was caused by metabolic processes due to the growth of the embryo in the egg. The metabolic activity of the embryo produces water vapor which is discharged through the pores of the eggshell (Ar and Rahn, 1980). According to Mortola *et al*. (2015) that water evaporation occurs due to the difference in partial pressure of water vapor between the inside (high concentration) and outside of the egg (low concentration). The sodium hypochlorite solution used in this study is a solution that has disinfection properties. Disinfection is used to inhibit or destroy microorganisms attached to the tooth surface (McNeme *et al*., 1991). Besides being used to inhibit contamination, hypochlorite can also thin the cuticle layer on the eggshell so that it plays a role in opening the pores of the eggshell (Harikrishnan, 2013).

Sodium hypochlorite is an oxygenating halogenated group, which is made from chlorine (Cl2), which has high level disinfectants, that is, it is very active on all bacteria, viruses, fungi, parasites, and some spores (David and Munadziroh, 2005). The use of this material is to prevent contamination from various bacteria which are often reported as the cause of low egg hatchability in artificial hatcheries (machines). The application of 0.25 percent sodium hypochlorite in the P2 treatment gave stable weight loss results in this study. This is in accordance with reports from David and Munadziroh (2005) in their report that sodium hypochlorite at a level of 0.5% is able to thin the acrylic layer on teeth after soaking.

Eggshell Temperature Fluctuations

Data on eggshell temperature observations during the hatching process are presented in Table 3. Table 3 shows changes in shell temperature during hatching from day 0 to day 21st of hatching. Shell temperature fluctuations occur as the hatching time increases (Alasahan and Copur, 2016; Hasyim *et al*., 2021). In this study, it can be seen that the average temperature on day 0 was 35.8°C, on day 7th it increased to 36.6°C, then again increased on day 14th to 38.3°C. In the last period of the 21 days of hatching the egg temperature decreased to 37.6°C.

The data obtained showed that the lowest shell temperature was recorded on the day 7th in treatment P1 (36.3ºC) while the highest was in treatment P3 (36.9ºC), while P2 showed a stable temperature. At the 14th age of hatching, the lowest temperature was recorded in treatment P2 (38.0ºC) and the highest was in P3 (38.8ºC). In the last period, namely the 21st age of hatching, the highest shell temperature was recorded, namely at P3 of 37.6ºC. Overall, the data shows that egg temperatures

Table 2. Weight loss egg

Treatments	Weight loss (g)			
	0 days	7th days	14th days	21th days
P1	46.35±4.15[a]	44.28±4.00[a]	41.96±3.92[ab]	39.14±3.69[b]
P2	46.34±3.72[a]	44.49±3.68[a]	42.25±3.67[a]	40.02±3.66[a]
P3	45.55±3.29[b]	43.52±3.25[b]	41.54±3.28[b]	39.54±3.38[ab]

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

P1: washing with warm water 40°C; P2: Washing with warm water 40°C + Concentration 0.25% hypochlorite; P3: Washing with warm water 40°C + Concentration 0.5% hypochlorite.

Table 3. Eggshell temperature fluctuations

Treatments	Eggshell temperature (°C)			
	0 days	7th days	14th days	21th days
P1	35.4±2.0[c]	36.3±0.5[c]	38.2±1.1[b]	37.4±0.7[b]
P2	35.9±0.4[b]	36.6±0.6[b]	38.0±0.7[c]	37.4±0.6[b]
P3	36.1±0.3[a]	36.9±0.5[a]	38.8±0.4[a]	37.6±0.5[a]
Average	35.8±0.9	36.6±0.5	38.3±0.7	37.4±0.6

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

P1: washing with warm water 40°C; P2: Washing with warm water 40°C + Concentration 0.25% hypochlorite; P3: Washing with warm water 40°C + Concentration 0.5% hypochlorite.

experience an increasing trend from age 0 to age 14, and in the last period at week 4 (21 days of hatching) the temperature decreases. This was because it entered the last period before hatching.

This fluctuation in egg temperature increase during hatching is in accordance with a research report from French (1997) that during the hatching process, the temperature of the egg embryo is below the temperature of the machine because the embryo's metabolism has not yet started. However, when the hatching process is underway, the embryo develops with metabolic processes that increase the temperature of the egg shell above the temperature of the machine. The decrease in temperature that occurs at the age of 21 is due to the eggs entering the hatcher period or the period before hatching to produce day old chickens (DOC) (Hasyim *et al*., 2021).

In the period of eggs entering the hatching period or the period before hatching, the temperature of the incubator is lowered, while the humidity of the machine is increased. This treatment also affects the temperature of the egg shells inside the machine. The decrease in temperature during this period aims to provide the optimal temperature so that the DOC can pipe and come out of the egg shell or hatch. In the research, the treatment given did not have a significant effect on decreasing shell temperature. The increase and decrease in temperature that occurs during hatching is due to the increased metabolic activity of the embryo (Hasyim *et al*., 2021; French, 1997), causing the release of heat through the eggshell Youssef *et al*. (2014). Additional reports from Sotherland *et al*. (1987) that the high temperature of the egg shell is caused by the metabolic activity of the egg embryo which produces heat and is released in the form of steam through the egg shell. However, the use of hypochlorite has not been able to increase heat release in eggs.

Fluctuations in the increase and decrease in egg temperature during hatching are the effects of the development of the embryo which is undergoing growth development in the egg. As a result of the growth of the embryo, it generates heat which is released through the eggshell. This is in accordance with the research of Lourens *et al*. (2007) that temperature has a different effect during the hatching process, high temperatures at the beginning of hatching will accelerate embryo growth in terms of utilization of nutrients and energy from the yolk and albumen, then these temperatures will decrease before the eggs hatch. The optimal temperature during hatching determines the metabolic rate of the embryo during incubation (Harun *et al*., 2001).

Hatchability

The hatchability parameter is the main indicator of success in hatchery activities. Hatchability is the percentage of eggs that successfully hatch from the total number of fertile eggs hatched. The effect of washing eggs using warm water plus hypochlorite solution at different concentration on hatchability is presented in Table 4.

Table 4 shows that treatment P2 resulted in a higher average hatchability of 82% compared to treatments P1 and P2, respectively 70% and 73%. The hatchability of P2 was 9% higher than the other washing treatments. Treatment P2 with hypochlorite level 0.25% washing showed better results than washing with warm water 40°C and 0.5% hypochlorite. The use of hypochlorite as a disinfectant aims to thin the thick cuticle layer on the eggshell and act as a disinfectant against bacterial and microorganism contamination (Harikrishnan *et al*., 2013). Microorganisms that are often found on the surface of hatching egg shells are thought to be the main cause of low hatchability in artificial hatching eggs (Reijrink, 2008). The results of Harrison's research (1969) that chicken hatching eggs that have been cleaned using a hypochlorite solution can produce a hatchability up to 89% compared to eggs without hypochlorite washing. The results showed that the use of a hypochlorite concentration of 0.25% was more effective for washing KUB chicken eggs than a concentration of 0.5%. This is presumably because the disinfectant properties contained in P3 are more concentrated so that apart from inhibiting contamination it also affects embryo development. This statement is supported by Zamzamy *et al*.

Table 4. The effect of washing on the hatchability of KUB chicken eggs

Treatments	Samples (n)	Hatch (items)	Hatchability (%)
P1	300	210	70
P2	300	246	82
P3	300	219	73
Average	300	227	75.6

P1: washing with warm water 40°C; P2: Washing with warm water 40°C + Concentration 0.25% hypochlorite; P3: Washing with warm water 40°C + Concentration 0.5% hypochlorite.

(2014), use of the correct dose of disinfectant will optimize the results of sanitation, because other than pathogenic microorganisms. The use of disinfectants also has an effect embryonic life.

Conclusions

The hatching index in this study was 76.71-77.38% and the three treatments were not significantly different. The weight loss in each period was P2, respectively 46.34 g (0 days), 44.49 g (7 days), 42.25 g (14 days) and 40.02 g (21 days). In general, egg temperature increased from age 0 to age 14, but in the last period at week 4 (21 days of hatching) the temperature decreased. The washing treatment of KUB chicken hatching eggs was not significant on the egg shape index, and had an effect on differences in egg weight loss and eggshell temperature. Egg washing with a hypochlorite concentration of 0.25% gave the highest hatchability of 82%.

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