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Egg hatchability of junglefowls (*Gallus gallus* Linnaeus) by natural method, artificial insemination and incubator

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Abstract The first experiment resulted that junglefowl naturally bred had significantly higher egg numbers than artificial insemination ($p < 0.05$), with an average of 34.29 ± 16.87 and 20.00 ± 5.41, respectively. Regarding the number of fertile eggs, it was found that the natural method was significantly lower than artificial insemination ($p < 0.05$) with an average of 12.94 ± 1.36 and 34.50 ± 12.04 eggs, respectively. The second experiment showed that hatching by natural method, which the hens incubated the eggs in the nest, the hatching rate was 78.57 ± 13.47%. Moreover, the eggs hatched in the incubator had the hatching rate of 67.43 ± 8.15%, of which, both methods had not statistically differed ($p > 0.05$). However, hatching in an incubator was better than orther methods as it shortened the incubation time of the laying hens. Consequently, it resulted in hens to be able to lay more eggs than hens that hatched naturally.

Keywords: Artificial insemination, Incubator, *Gallus gallus gallus*, Hatching rate, Junglefowl

Introduction

Red junglefowls (*Gallus gallus* Linnaeus) in Thailand are classified as protected wildlife under the Wildlife Preservation and Protection Act 1992 (of Thai B.E. 2535). There are two subspecies of junglefowls in Thailand: the white-eared and the red-eared. The general characteristics of a junglefowl usually has golden-orange and bronze-red upperparts, red comb and wattles, blue-green, rufous, and brown-black underparts, large iridescent, green-black tail, white feather patch at tail base, and gray legs and feet. The difference between the two subspecies which the white-eared has the appearance of a long neck feather and the flesh around the earlobe is large with white specks. In the red-eared, the neck feathers have medium length. The flesh around the earring is small and often red (Dorji *et al.*, 2012).

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White-eared junglefowls are native in the countries like Thailand, Cambodia, and Vietnam. In Thailand, junglefowls are found in the northeast, from the Mekong River, Sakon Nakhon, Ubon Ratchathani, Surin, Sisaket, and Prachinburi, to the eastern part such as in Chanthaburi and Trat provinces. Junglefowls live in bamboo forests, dry evergreen forests, deciduous dipterocarp forests, and mixed deciduous forests from the ground level to an altitude of 1,800 m. Junglefowls feed on mostly seeds from grass, bamboos, and fallen fruits. They also feed on insects, worms, and other small animals in nature to maintain the balance of the forest. When searching for food, they go in small flock in the early morning, on the forest floor. During the mating season, the male junglefowls mate with females within his flock and protects them from rivals. Male junglefowls announce their presence through crowing as a territorial call, just before mating, or to show dominance over others in the flock. Females could lay for about 5-10 eggs with an incubation period of 21 days (Boonsanong, 2009).

However, the number of junglefowls in the forest has rapidly decreased due to human invasion on the hunt for food and its habitat loss. With this imminence, it is necessary to study the cultivation of such junglefowls. Although incubating eggs under natural conditions has an advantage of high hatching and survival rates, a cost-saving method of raising chicks, the hens would be able to lay only a clutch of eggs in one season after hatching. These hens will then start caring to their chicks (Boonsanong, 2002). According to the 1992, Wildlife Conservation and Protection System in Thailand, junglefowls, or wild chickens, has allowed to cultivate, raise, and propagate to be released back to its natural habitat. Thus, this study aimed to compare the hatching rate of junglefowls's eggs by natural method and with the use of electric incubators to preserve junglefowl breeds from extinction. In addition, this study was to investigate good consciousness in maintaining a natural balance and to create a way to increase biodiversity for the conservation of resources in the system.

Materials and methods

This study was conducted from October of 2019 to September 2020, at the Department of Animal Production and Technology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-ok Chanthaburi Campus in Thailand.

Experimental animals

Experiment 1: Comparison of hatching rate between wild fowls that were bred by natural method and artificial insemination. The used Junglefowls

were consisted of 4 males and 10 females, in the reproductive stage, healthy, and vaccinated against disease prior to trial in accordance to the Animal Disease Prevention Control Program by the Department of Livestock Development of Thailand (Boonsanong, 2009). These animals were divided into 2 groups: natural method and artificial insemination.

Experiment 2: Comparison of hatching rate of junglefowl eggs hatched by natural method and in the incubator was investigated. The used Junglefowls were consisted of 5 males and 10 females, in the reproductive stage, healthy, vaccinated against disease prior to trial in accordance to the Animal Disease Prevention Control Program by the Department of Livestock Development of Thailand (Boonsanong, 2009). These animals were divided into 2 groups as natural method and hatched in the incubator.

Management and feeding of experimental animals

A breeder cage of 2.5 × 5 × 2 m that had an indoor section of 2.5 × 2 m^2 with floor covered with coarse sand that was about 5 cm thick and an outdoor section of 2.5 x 3 m^2, and kept the animals. The condition was sandy and grassy to let the jungle fowls get some exercise. The cage had a perch of about 1.50 m high for sleeping, and a laying nest made from bricks topped with grass at the inner corner. Feed for laying hens was comprised of no-less than 15% of protein and whole grains (*Khao Yai* feed). Other supplements such as worms, fruits (from time to time), and multivitamins mixed water (every other day) were also given to the chickens. Feeding time was at 06.00-07.00 h. Feed and water were taken away from the cage every evening to prevent from rodent infestation and were then given a new set of feed by next morning.

Data collection

Experiment 1 was recorded the number of eggs which obtained in each group and its weight before hatching. At 7 days of incubation, candling of eggs in different groups were done using a flashlight to check which eggs were fertilized or not. On the 14^{th} and 18^{th} day after the eggs were incubated. A test was performed to screen out unfertilized eggs. The infertile eggs, if left for a long period of time in the incubator, may explode and should be removed to avoid laying hens leave its nest. The number of hatched eggs for analyzing the hatching rate was likewise recorded. Experiment 2 were gatheret data as egg weight, egg width, and egg length in each group before hatching. At 7 days of incubation, candling of eggs in different groups were done using a flashlight to check which eggs were fertilized or not. The number of hatched eggs for analysing the hatching rate was also recorded.

Data analysis

Statistical data were analysed according to two representative comparison trial plans: analysis of variance (ANOVA) and Proc. GLM (SAS, 1985); and then compared the average of each treatment.

Results

Egg Number, egg weight, and hatching rate between junglefowl bred under natural method and artificial insemination

It was found that junglefowl that bred naturally had significantly higher egg numbers than artificial insemination ($p < 0.05$), with an average number of 34.29 ± 16.87 and 20.00 ± 5.41, respectively, but the egg weights of under both mating methods were not statistically different ($p > 0.05$). Regarding the number of fertile eggs, natural method had significantly lower than artificial insemination ($p < 0.05$), with a mean of 12.94 ± 1.36 eggs and 34.50 ± 12.04, respectively. "Dead germ" eggs from both mating methods were not statistically different ($p > 0.05$), but the hatching rate of natural mating was significantly higher than artificial insemination, with an average of 87.0.6 ± 1.36 and 65.50 ± 12.04, respectively (Table 1).

Table 1 Comparison of egg number, egg weight, and hatching rate between junglefowl bred under natural method and artificial insemination

Factor	Natural method	Artificial insemination	value-P
Egg number(%)	16.87 ± 34.29	± 20.00 5.41	0.049*
Egg weight (g)	34.90 ± 0.73	35.50 ± 2.05	0.070
Fertile egg (egg)	12.94 ± 1.36	34.50 ± 12.04	0.001**
Dead germ eggs (egg)	12.18 ± 10.79	4.00 ± 8.94	0.726
Hatching rate(%)	87.06 ± 1.36	65.50 ± 12.04	0.001**

* **:Remark**= statistically different at a significant level of 0.05

** =statistically different at a significant level of 0.01

Hatching rate of junglefowl eggs hatched by natural method and in the incubator

The junglefowl eggs had a mean egg width of 34.29- 34.48 mm and egg length of 46.57-46.60 mm. The average weight was 24.56- 24.73 g. The morphology of junglefowl eggs that were used to compare the hatching rate under natural and incubator hatching which was not statistically different ($p > 0.05$) (Table 2).

Hatching under natural method was done where the hens incubated their eggs in the nest with rice straw as the nesting material. By comparing the percentage of hatching rates, the natural method was 78.57 ± 13.47% , while under an incubator was 67.43 ± 8.15%. Moreover, the eggs hatched in the incubator used for 21-day incubation period as with the natural method. Both methods were not statistically different ($p > 0.05$).

Table 2 Comparison of egg weight, egg size, and hatching rate of junglefowl eggs hatched by natural method and in the incubator

Factor	Natural method	Incubator	value-P
Egg weight (g)	0.96 ± 24.73	0.47 ± 24.56	0.191
Egg width (mm)	34.29 ± 34.48	34.48 ± 0.84	0.204
Egg length (mm)	0.88 ± 46.57	1.01 ± 46.60	0.789
Hatching rate (%)	13.47 ± 78.57	8.15 ± 67.43	0.354

*** :Remark**= statistically different at a significant level of 0.05
** =statistically different at a significant level of 0.01

Discussion

Based on the results, it was found that the number of eggs of red junglefowls under natural method was higher than those by artificial insemination ($p < 0.05$). The hatching rate from natural method was also higher ($p < 0.05$); however, the artificial insemination gave a higher rate of eggs with embryo than natural method ($p < 0.05$). According to Anwar *et al.* (2016), reproductive ecology of the junglefowls (*Gallus gallus murghi*) in Deva Vatala National Park in Pakistan found that an average number of 3 baby chicks in each nest. Reproductive behavior of those junglefowls was peak in June and July it was rainy season as there was plenty of food such as crops and a large number of small insects. The junglefowls had their reproductive behaviors as harem with a ratio of male chicken to female chickens (1:3). After breeding, 4-7 eggs would be found in each nest. Zakaria (1999) mentioned that if the junglefowls live in proper places rich in food especially in rainy season, the hatching rate was more than 90%. In this study, the results showed that the hatching rate of the red junglefowlsunder natural method was 87.06 ± 1.36% which indicated that the rate of hatching would be high or low depending on the richness of food. As supported by Nolte *et al.* (2021), the quantity of nutrition and quality of food had direct impact on quality of eggs.

Similarly, Saeki and Inoue (1979) reported that a number of 18 female junglefowls inseminated artificially (AI) could lay 28 eggs, with 1 egg per year in average. Healthy male and female breeders should have aged up to 298 days and weigh around 887 g, or more. The eggs weighed approximately 33.4 g.

While crossbreeding of Gallus junglefowls with White leghorns showed that 52 female chickens could lay approximately 147-183 eggs per year in average. Suitable breeders should have aged up to 163-182 days and weigh around 1,259-1,347 g, or more. Their eggs weighed approximately 33.1-37.2 g. In comparison to this study, the results indicated that AI was a good choice to breed the junglefowls because the number of eggs with embryo were higher than eggs from natural method ($p < 0.05$) In addition, AI could be used to crossbreed between junglefowls and White leghorns which could evidently give a higher number of eggs.

The morphology of red junglefowl's eggs under natural method gave an approximate width of 34.29-34.88 mm; the average length was 46.57-46.60 mm while the weight was around 24.56-24.73 g. When comparison with the Gallus junglefowl's eggs in Japan (Saeki and Inoue, 1979) to the results which found that the Gallus junglefowls in Thailand produced smaller eggs than the former, which had an average weight of around 34.8 ± 3.0 g. When comparing weight of the eggs in this study with the crossbreeding eggs between Gallus junglefowls and White leghorn chickens, the results showed that weight of the eggs from red junglefowls was lower than eggs from crossbreeding. The average weight of both types of eggs were 24.56-24.73 g and 46.7-48.2 g, respectively. The size of red junglefowl's eggs were noticeably smaller than White leghorn chickens' eggs. The latter had an average weight of around 57.4 g. According to Kerje *et al.* (2003), the weight of eggs from red junglefowls grown in tropical areas and those grown in cold places were similar. It was found that size of eggs from junglefowls grown in Thailand and Thai junglefowls grown in a zoo in Sweden were comparable as the average weights were around 24.56-24.73 g and 23.0 g, respectively (Kerje *et al.*, 2003).

Moreover, Boonsanong and Prasertsan (2004) reported that length and width of the eggs from red junglefowls had an average width around 34.29-34.48 mm and an average length of 46.57-46.60 mm which smaller than the eggs from Thai Phasianidae chickens. The Lophura pheasants had an average width of eggs of about 38.28-39.63 mm and an average length of 51.36-52.97 mm. The Syrmaticus pheasants had an average width of eggs around 33.48 mm and the length was approximately 44.11 mm. The Burmese red junglefowls (*Gallus gallus spadiceus*) had averaged width of eggs of 34.04 mm and the length was around 44.65 mm. According to the research of Boonsanong (2002, 2004) stated that the color of eggs from the Burmese red junglefowls had neither any impact on the hatching rate. The hatching period was 19 days and a baby chick weighed was 18.42 g. A baby Burmese red junglefowl on its 5^{th}-6^{th} week was able to observe its sex; and as it reached its first year of age, could start breeding (Boonsanong, 2002).

The number of clutches of Gallus junglefowls in each country were various as following: the clutch size in Pakistan was around 6.1 ± 2.3 (Anwar *et al.,* 2016); it was 5-7 in India (Bump and Bohl, 1961); 2-12 in Malaysia (Nishida *et al.,* 1975); the size in Myanmar was 5-6 (Meijer and Siemers, 1993); and it was 6-8 in Thailand as Boonsanong (2009) who stated that the clutch size of Phasianidae pheasants in Thailand were in the same family as junglefowls. Therefore, hatching in an incubator could help the clutch to continuously produce larger number of eggs compare to natural hatching. The study of Romanov and Weigendt (2001) and McBride *et al.* (1969) found that the Gallus junglefowls naturally laid 10-15 eggs per year and hatching rate was about 4-6 chicks only. In terms of hatching a large number of Phasianidae chickens, hatching in an incubator was better because if female junglefowls hatched their eggs themselves, they would not lay any new set of eggs. However, if the eggs were taken to hatch in an incubator, this would help the female junglefowls to continuously lay eggs until the end of laying period. Moreover, air in an incubator flowed all the time and the temperature was stable, so the temperature and humidity could be easily controlled. Hatching in an incubator employed the same principles as natural hatching. The machine was designed to control temperature and humidity as same as the temperature from female chickens. The eggs were flipped in order to receive warmth thoroughly and hatching period was as same as natural hatching (Boonsanong, 2009).

In summary, the incubation rate of junglefowl eggs by natural method and in the incubator was not statistically different ($p < 0.05$). However, hatching in an incubator was better as it shortened the incubation time of the laying hens. Consequently, it resulted in hens that able to lay more eggs than hens that hatched naturally.

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