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Original Research Paper

Eggs Fertility, and Hatchability of Kampong Hens Inseminated Using Brahma, Cochin, and Bangkok Roosters Semen

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Abstract: The objectives of this research were to evaluate Brahma, Cochin, Bangkok roosters spermatozoa quality and compare fertility and hatch-ability following insemination into Kampong hens. The semen quality was evaluated using a completely random design of three strains of the rooster with 5 replications. The results showed that spermatozoa concentrations of Brahma, Cochin, and Bangkok rooster were 1252.0± 7.92x106, 1140.0± 5.83x106, and 1304.0± 12.28x106 respectively ($p > 0.05$). The percentage of motility of Brahma, Cochin, and Bangkok spermatozoa was 72 ± 2.74 %, 74 ± 2.24 %, and 73 ± 2.74 % respectively ($p > 0.05$). The percentage viability of spermatozoa of Brahma, Cochin, and Bangkok were 99.2 ± 1.30 %, 99.4 ± 0.55 % dan 99.2 ± 1.10 % respectively ($p > 0.05$). The percentage normality of Brahma, Cochin, and Bangkok spermatozoa were 94.6 ± 2.30 %, 94.4 ± 2.70 % dan 97.4 ± 2.07 % respectively ($p > 0.05$). Fertility of eggs inseminated using Brahma, Cochin, and Bangkok, spermatozoa to Kampong hens were, 36.21 ± 17.52%, 34.29 ± 14.50%, and 73.27 ± 9.90% respectively and they were significantly different ($p < 0.05$). The hatchability of eggs inseminated using Brahma, Cochin, and Bangkok spermatozoa were 59.40 ± 34.80%, 87.26 ± 8.81%, and 82.63 ± 5.34% respectively and they were significantly different ($p < 0.05$). It can be concluded that Brahma, Cochin, and Bangkok roosters have good quality semen, which can be used inseminate into Kampong hens.

Keywords: Artificial insemination, crossbreed, hatching, spermatozoa.

Introduction

Kampong chicken under farmer's condition in Lombok Island was found relatively slow growth, consequently, potencies of this chicken as sources of protein were relatively small compared to broiler. On the other hand, the price of Kampong chicken meat is higher than that of broiler at the same weight, because Indonesians get used to the texture, taste, and flavor of Kampong chicken (Asnawi 1997). By having lower growth rates, to get the same weight, Kampong chicken needs to be raised longer than broiler. To get 1 kg body weight, kampong chicken needs more than 16 weeks, while broiler commercial only needs 3 weeks. Many efforts have been performed to increase Kampong chicken meat production, by improving management and genetics. One effort was to improve genetically by crossing between strains (North and Bell, 1990). T

hey reported that crossing between high and low eggs production strains would be in between both strains. However, the crossing effect may show higher than their parent's performance (Parkhurst and Mountney, 1987), because there was heterozygosity covered by a recessive gene. For that reason, North and Bell (1990) suggested that to improve genetically, chickens should be crossed between two strains, which brought genetic traits to be wanted. The results of Asnawi (1997) research showed that crossbreed between Kampong

rooster with broiler parent stock produced better production performance than that of Kampong chicken itself. In addition, he reported that this crossing showed phenotype as broiler, however, aroma, texture, and taste were closer to Kampong chicken.

Recently, chickens strains that grow faster than Kampong chicken available in Lombok Island are Brahma, Cochin, and Bangkok. This Brahma chicken was originally from India, with bodyweight up to 10 pounds for a young rooster, and mature rooster up to 12 pounds, as well Cochin was originally from China, mature chicken may reach the weight of 10 pounds (Department of Animal Science Oklahoma State University, 2021), while Bangkok was originally from Thailand with an adult body weight of up to 5 pounds.

The size and meat of these chickens showed compact muscle structure and were favorable to local people. Those three roosters are large strains, while Kampong chicken is a small strain, this would be difficult for crossing by natural mating and insemination would be the method of choice to overcome the mating problem. The objectives of this study were to evaluate the semen quality of Brahma, Cochin, and Bangkok roosters and to compare fertility and hatchability following insemination using those three strains of roosters semen to Kampong hens.

Materials and Methods

Experimental site

This research was conducted at the teaching farm Faculty of Animal Sciences, University of Mataram. Evaluation of rooster semen was performed in Immunology Laboratory, University of Mataram Indonesia.

Materials and Research Tools

Three mature roosters, with a bodyweight of more than 3 kg were used in the present study, those were Brahma, Cochin, and Bangkok rooster strains. They were placed in the pen of 1 m^2. Kampong hens of 15 hens with an average body weight of 1037.33 ± 50.21 g were placed in individual pens, fed with commercial feed mixed with rice brand and milled corn. The roosters were placed in a postal pen with feed and water were given ad libitum, twice a day in the morning and the evening. Semen collection and insemination were performed by using methods reported by Asnawi *et al*. (2021).

Semen collection and evaluation

A semen collection of roosters were accomplished by putting a plastic cup Millipore to the covered outside cloaca, with elastic string around the base of wings, to prevent loss during mating. The roosters were trained to get used to the spermatozoa collecting equipment. Semen collection was performed by holding the teaser on the ground with two hands on the base of hen wings and letting the rooster stand and mate the hen. Collected semen on the Millipore cover cup was then evaluated macroscopically soon after collection and microscopically in the laboratory, as reported previously (Asnawi *et al*, 2021)

Semen evaluation was performed by visual evaluations such as volume, color, and microscopic evaluations such as sperm concentrations, motility (%), morphology, abnormality (%), and viability, as reported by Ax *et al*. (2000); Peters, *et al*. (2008), Arifiantini (2012), and Asnawi *et al*, (2021). Macroscopic evaluation such a**s** volume of semen was measured by using 1 ml graduated syrinx. The Color of semen was evaluated visually such as creamy white, opaque, or between opaque and creamy white. The pH semen was evaluated by using pH *special indicator paper* (Merk, scale of 6.4 – 8). Semen of 5µL was dropped into pH paper and let for 15-30 seconds then the color change was compared to the standard.

Microscopic evaluation such as semen concentration was evaluated by direct cells count method using standard hemocytometer counting blood cells (Ax *et al*. 2000; Peters, 2008; Arifiantini, 2012 and Asnawi et al, 2021). It was performed by drawing semen into a dilution pipette to the 0.05 mark, and the pipet filled with hypotonic solution up to 101 marks. The hypotonic solution consists of 50 ml distillate water, 1 ml 2% eosin, and 1 ml 3% sodium chloride. The counting chamber was then filled and left to settle for 5 minutes before being placed into counting chambers. Spermatozoa were counted in 5 large squares in both chambers. The concentration of spermatozoa was calculated by multiplying the mean count by 10^7.

Assessment of motility was performed by percentage progressive motile spermatozoa or

moving forward direction. Semen was diluted until individual spermatozoa can be observed after placing a drop on the pre-warmed slide under coverslips and examined under 400x magnification.

Live and death spermatozoa were evaluated by counting spermatozoa on a slide after staining with nigrosine eosin. Five drops of stain were added to a drop of semen in pre-warmed object glass and smeared on slides and allowed to air dry before being evaluated under a microscope, all spermatozoa absorbing the stain were classified as dead, while heading of spermatozoa unstained considered as live spermatozoa within 200 sperm count. Morphology evaluation was performed by classifying 200 spermatozoa stained by nigrosine eosin, as either normal or abnormal such as malformed head, malformed tail, protoplasmic droplet, and decapitated spermatozoa. (Alkan *et al* 2002).

Insemination

Insemination was performed using $100x10^6$ normal, life, and motile spermatozoa were estimated by volume (ml) concentrations (x 10^6), motility (%),normality (%) divided by $100x10^6$. Then the final volume of extending semen was calculated by several insemination time volumes of insemination (0,2 ml). The semen was then added extender up to final volume (as reported by Asnawi et al 2021) insemination was performed every second day.

Eggs collection and selection

Eggs were collected on the second day after the first insemination, then followed every day for a week. The eggs were selected by smooth shell and uniformity size, the selected eggs were then cleaned, disinfected, and incubated using an automatic incubator. Egg's fertility and Hatchability. Eggs fertility was evaluated by scooping in a dark room 3 days following incubation and eggs fertility was calculated by:

$$Fertility = \frac{(n)\ incubated\ eggs - (n)\ fertile\ eggs}{(n)\ incubated\ eggs}\ X\ 100 \quad \textbf{(1)}$$

Eggs hatchability was evaluated by several hatched eggs following incubation and calculated by:

$$Hatch\ ability = \frac{(n)\ hatched\ eggs}{(n)\ fertilized\ eggs}\ X\ 100 \quad \textbf{(2)}$$

Hatch weight was performed by weighing the newest hatched chicken using the O'hause scale soon after hatch, by observing the incubator in the morning, afternoon, and evening.

Experimental Design

The experiment was designed using a Complete Randomized Design which consists of three treatments: Those treatments were crossing between Kampong hens with Bangkok rooster (R-1), with Brahma rooster (R-2), and with Cochin rooster (R-3). Each treatment consisted of one rooster and fifteen hens. Each treatment was replicated four times and 50 eggs from each treatment were incubated to evaluate their fertility and hatchability.

Parameters Measured

Parameters measured in the present study were 1). Macroscopic semen evaluation such as volume, color, consistency, odor, and pH. 2). Microscopic spermatozoa evaluation such as viability, motility, and morphology of spermatozoa. 3. Evaluation of egg fertility and hatchability following insemination.

Statistical Analysis

The Complete Randomized Design was used in this analysis by comparing three strains of rooster those were Brahma, Cochin, and Bangkok. Each with five replications and the data was evaluated by analysis of variance.

Results and Discussion

The results of the macroscopic evaluation of volume, color consistency, odor, and pH of Brahma, Cochin, and Bangkok rooster are presented in Table 1. The volume of semen collection from Brahma, Cochin, and Bangkok roosters was found significantly different (p<0.05), and the volume of semen Bangkok rooster was found higher than that of Brahma and Cochin, the reason for this is unclear. It can be speculated that it may be because Bangkok chicken has adapted to the Indonesian environment longer than Brahma and Cochin. Factually Bangkok chicken has been brought to Indonesia decades ago, while Brahma and Chosin chicken was found in Indonesia for the last several years. In line with these

results, it was reported (Sonseeda *et al*, 2013) that chicken that well adapted to the environment had sperm quality better than the imported rooster.

It was also speculated that semen volume in the present study may be influenced by strain or breed of chicken, Hrnčár (2013) reported that ejaculate volume influenced by strains or breed, it was found that Brown Leghorn rooster has ejaculate volume higher than Brahma, it was found that Brahma semen ejaculate volume was 0.49 cm^3 while volume semen Brown Leghorn cocks was 0.73 cm^3. Volume Bangkok semen was in between results in two different strains reported by Jarinkovičová (2012), that volume ejaculate of Barred Plymouth-Rock was 0.66 ml and Light Sussex was 0.46 ml. However, the semen volume of three strains in the present study is lower than the semen Rhode Island Red (RIR) rooster which is up to 0.87 ml (Máchal 1999). Another study (Tesfay *et al*. 2020) found that semen volume of RIR was 0.52 ± 0.03 mL and White Leghorn was 0.24 ± 0.02 ml which may compare to the results in the present study.

Table 1. Visual evaluation of semen characteristics of Brahma, Cochin, and Bangkok roosters

Visual evaluation	Rooster strains		
	Brahma	**Cochin**	**Bangkok**
Volume (ml)	0.36 ± 0.09^a	0.32 ± 0.04^a	0.52 ± 0.04^b
Color	creamy white	creamy white	creamy white
Consistency	viscous	viscous	viscous
Odor	specific	specific	specific
pH	6.95±0.31	6.97±0.25	6.98±0.25

Note: Value with the different superscript showed significantly different (p<0.05).

In comparison with local roosters, the semen volume in the present study is comparable to Sentul rooster which was found 0.50 ± 0.15 ml (Setiadi *et al*, 2017), and higher than that of IPB–D1 rooster of 0.10±0.07 ml (Setiadi et al, 2019) and higher than mixed local chicken of 0.24±0.12 mL (Hambu *et al*. 2016). It was also reported that the volume of semen can be influenced by physiological effects, process spermatogenesis, and methods of semen collection (Tarif *et al*. 2013, MKPUGHE, *et al*., 2015). Other researchers (Donoghue *et al.* 2000) showed that ejaculate volume depends on strain or breed, ages, individual, season, light, and other environmental factors.

The results of the evaluation of motility, viability, normality, and concentration of spermatozoa were presented in Table 2. The results showed that the percentage of sperm motility of Brahma, Cochin, and Bangkok roosters was not significantly different (p>0,05). The motility in the present study was higher than that reported by Hrnčár (2013) who found that the motility of spermatozoa of Brahma, Leghorn, and Oravka were 57.51%± 20.28, 64.48 ±18.79%, and 62.25 ±19.01% respectively. On the contrary, the result of the present study was lower than that of Rhode Island Red which was reported to have spermatozoa motility of 85.5 to 88.3% (Máchal et al 2002), Sentul of 88.05 ± 2.07% (Saleh *et al*, 2017), Local chicken of 82.5% (Nugroho dan Saleh, 2016) another Local chicken 77.57 ± 3.67% (Lubis, 2011), and lastly Bangkok of 78.9 ± 1.92 % (Hijriyanto *et al*, 2017).

Table 2. Microscopic evaluation of spermatozoa characteristics of Brahma, Cochin, and Bangkok roosters

Microscopic evaluation	Rooster strains		
	Brahma	**Cochin**	**Bangkok**
Motility (%)	72 ± 2.74	74 ± 2.24	73 ± 2.74
Viability (%)	99.20 ± 1.30	99.40 ± 0.55	99.20 ± 1.10
Normality (%)	94.60 ± 2.30	94.40 ± 2.70	97.40 ± 2.07
Concentration x 10^6/ ml	1 252.0 ± 7.92	1 140.0 ± 5.83	1 304.0 ± 12.28

Note: Value with the different superscript showed significantly different (p<0.05).

Evaluation of percentage of viability semen Brahma, Cochin, and Bangkok rooster was presented in Table 2, and this viability was not significantly different statistically (p>0.05). Viabilities of spermatozoa were higher than that of local rooster which was found 83.87 ± 2.22% (Lubis, 2011), Kedu rooster 86.99 ±2.14% (Saleh *et al*, 2017), Kedu rooster 91.45±6.30%, Local 91.05±6.30% and Merawang roosters 90.02±

7.84% (Hambu *et al*. 2016). Hermiz *et al*, (2016) reported that viability of local and Isa Brown crossed rooster was found 93.08%. Also, it was reported by Tarif *et al*. (2013) that the proportion of live spermatozoa was varied from 82.20% to 87.30% between strains. The results of the viability of spermatozoa in the present study were considered better than in another study previously.

Evaluation spermatozoa morphology was used to evaluate the percentage of normality of spermatozoa. The results of normality spermatozoa of Brahma, Cochin, and Bangkok in the present study were presented in Table 2, statistically, the analysis showed non-significant differences (p>0.05) between them. It was reported that normality spermatozoa depend on the abnormality of spermatozoa, such as abnormality primer which occurs during spermatogenesis, while abnormality secondary which occurs during maturation of spermatozoa. It was reported that abnormalities were not influenced by strain or breed (Sonseeda *et al*, 2013), but it influenced by ages, the protein content of the feed, vitamin E, and calcium contents (Selvan, 2007). As a comparison, the study performed in four breeds of chicken (Black Minorca, Green-Legged, Italian Partridge Partridge, and White Crested Black Polish) showed semen quality was not significantly different (Siudzinska and Lukaszewiez, 2008).

Evaluation of spermatozoa concentration of Brahma, Cochin, and Bangkok rooster was presented in Table 2 which is statistically not significantly different (p>0.05). This evaluation of spermatozoa concentration was not influenced by strain or breed, a similar finding was reported by Hrnčár *et al.* (2013), although with spermatozoa concentration lower than that the results in the present study. They found that Brahma, Leghorn Brown, and Oravka rooster with spermatozoa concentrations were 1.91±0.44 x10^6/mm^3, 2.59±0.50 44 x 10^6/mm^3, and 2.52±0.47 44 x 10^6/mm^3 respectively. On the contrary Malik *et al.* (2013) and Hermiz et al. (2016) found that the concentration of spermatozoa varied between breeds. Malik *et al,* (2013) found that spermatozoa concentration of Red Jungle fowl, Domestic Roosters, and Bantam Roosters, were 4 440±9.05 × 10^6/ ml, 2 730±10.5 × 10^6/ml, and 1 830±7.43 × 10^6/ml respectively, while Hermiz *et al.* (2016) reported that spermatozoa concentration varied between rooster from 3 650 × 10^6/ ml to 5 890 ×10^6/ ml. Another study by Tarif *et al.* (2013) found that spermatozoa varied between roosters with a range from 9 600×10^6/ ml to 7 500×10^6/ ml. The variation of spermatozoa concentration was influenced by many factors such as feeding, size, and body weight (Malik *et al.*, 2013) seasons (Elagib *et al*., 2012). Finally, Donoghue *et al.* (2000) found that spermatozoa concentration increases by increasing body weight.

The results of the present study found that egg weight between three rooster breeds was not significantly different (p>0.05). It can be seen from Table 3, that egg's weight range is between 38 gr and 40 gr. These results are lower than that reported by Argo *et al* (2013) and Yuma et al (2014), they found that the average eggs of Arabic Silver and Gold each were 42.75±2.22 gr and 46.81±2.41 gr respectively. Eggs weight in the present study was higher than the results study of Indra et al (2013), who found eggs weighed between 30-35 grams. The uniformity of eggs weight in the present study may be because the age of Kampong chickens used was relatively uniform age between 8 -9 months and weight between 1130-1140 gr. Bell and Weaver (2002) reported that many factors may influence egg weight such as the age of hens, environment temperature, strain or breed, nutrition quality, bodyweight of hens, and time of laying eggs. They also reported that other factors which may reduce egg weight were low protein, calcium, vitamin D, salt, and iron in the feed ration.

Egg's fertility of kampong hens inseminated with Brahma, Cochin, and Bangkok spermatozoa are presented in Table 3. Fertility results of eggs inseminated with Bangkok rooster were higher than that of Brahma and Cochin roosters. The reasons that the Bangkok rooster has higher fertility than Brahma and Cochin in the present study, was not understood. However, it was a fact that Bangkok chicken has been brought earlier and consequently has been adapted to local environments longer, as Brahma and Cochin chicken was only recently being brought and rise in Lombok Island. The previous study (Hrnčár et al, 2015a) showed that the fertility of Brahma, Chocin, and Orpington was lower than that of the present study, with the fertility of 59.81%, 57.36%, and 58.81% respectively. Another study (Stanley *et al*, 2012) showed higher fertility of Cochin chicken was found up to 85%. It was much higher than that of Cochin eggs fertility in the present study.

Table 3. Egg's weight, fertility, hatchability, and hatching weight crossing kampung chicken with Brahma, Cochin, and Bangkok rooster

Variables evaluated	Rooster strains		
	Brahma	Cochin	Bangkok
Eggs weight (gr)	40.07 ± 0.27	39.87 ± 1.10	38.38 ± 0.67
Fertility (%)	36.21 ± 17.52[a]	34.29 ± 14.50[a]	73.27 ± 9.90[b]
Hatchability (%)	59.40 ± 34.80[a]	87.26 ± 8.81[c]	82.63 ± 5.34[b]
Hatch weight (gr)	34.42 ± 0.61	34.20 ± 2.75	33.45 ± 3.42

Note: Value with different superscripts in the same rows differs significantly ($p< 0.05$).

The results of the percentage of hatchability of crossing between Kampong hens with Bangkok, Brahma, and Cochin rooster are shown in Table 3. It showed that the higher hatchability in the present study crossing with Brahma, Cochin, and Bangkok roosters were 59.40 ± 34.80%, 87.26 ± 8.81%, and 82.63 ± 5.34% respectively. The reason that the percentage of hatchability in the present study that the Brahma rooster was lower than that of the others was not understood and unexplainable. However, the hatchability of eggs Cochin and Bangkok crossing were comparable to the results of Hrnčár et al (2015a), who found that the average hatchability of Brahma, Cochin, and Orpington chicken were 80.08%, 82.54%, and 89.86% respectively.

The result of hatch weight in the present study was between 33 to 34 gr. This hatch weight is lower than the results of crossbreeding of Bangkok roaster with commercial laying hens which were found 38.49 ± 0.43 gr (Badaruddin et al. 2017) and higher than that of results Arab hens of 27.37 to 28.80 gr (Susanti et al., 2015), Black Kedu of 28.97 gr (Nataamijaya, 2008), White Kedu of 25.5 gr, and Sentul Chichen of 32.2 gr (Hidayat and Sopiana, 2010), and Pelung of 31.83 gr (Darwati, 2000). According to North and Bell (1990), there is a high correlation between the weight of eggs and hatched weight. In line with this Hasan et al. (2005) stated that the greater the weight of eggs would produce the greater day-old chicken weight. This hatch weight found in this study was considered as the normal weight of kampong chicken.

Conclusion

It can be concluded that spermatozoa quality of Brahma, Cochin, and Bangkok rooster were not significantly different, however, Bangkok semen volume was higher than that of Brahma and Cochin. Egg's fertility of crossing between Kampong hens and Bangkok rooster was higher than that of crossing with Brahma and Cochin roosters. Finally, artificial Insemination can be practiced in Kampong chicken using Brahma, Cochin, and Bangkok roosters spermatozoa.

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