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ABDUL MALIK

ABD WAHID HARON

ROSNINA YUSOFF

M NESA

MUHAMMAD BUKAR

See next page for additional authors

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Abdul MALIK^{1,2}, Abd Wahid HARON^{2*}, Rosnina YUSOFF², M. NESA², Muhammad BUKAR², Azhar KASIM³

¹Department of Animal Science, Faculty of Agriculture, Islamic Kalimantan University, Banjarmasin, Indonesia

²Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia

³Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia

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Abstract: The objective of this study was to investigate the semen quality of 3 chicken breeds: the red jungle fowl, domestic chicken, and bantam chicken (*Gallus gallus domesticus*). A total of 27 cocks, including 9 cocks each of red jungle fowl, domestic chicken, and bantam chicken, were used in this study. Semen was collected once a week by dorso-abdominal massage method. The semen was evaluated for volume, concentration, motility, live/dead ratio, and percentage abnormalities. There were no significant differences in the semen volume and general motility among the 3 breeds. However, the semen concentration was significantly different between the red jungle fowl (4.44×10^9 sperm/mL) and bantam chicken breeds (1.83×10^9). The percentage of forward motility in the red jungle fowl was significantly higher than that of the domestic chicken and bantam chicken, while the percentage of spermatozoa with rotating motility was significantly higher in the bantam chicken and domestic chicken. It was concluded that the semen concentration, individual motility, and total abnormalities were significantly different among bantam chicken, domestic chicken, and red jungle fowl and the semen volume and concentration was highest in the red jungle fowl.

Key words: Semen collection, semen evaluation, semen quality, red jungle fowl, domestic chicken, Malaysian bantam chicken

1. Introduction

Selection of males for breeding programs is different between avian and mammalian species. In mammals, the testes are located outside the body, while in poultry, the testes are internally located within the body. Thus, breeding soundness evaluation in poultry does not include examination of the scrotum but must instead rely on macroscopic and microscopic examination of the spermatozoa to determine fertility of the cock. Reproduction in the jungle fowl is a complex mechanism with multiple environmental and physiological factors interacting and contributing to successful copulation and fertilization. For a cock to be successful, it must be physically able to copulate and transfer semen to the female. Furthermore, fertilization in poultry depends on age, sperm selection, timing between mating, oviposition, and sperm quality (1). Rowe et al. (2) reported that ejaculate quality was assessed in terms of sperm numbers and sperm quality, whereas the samples of ejaculate were collected using a technique for massage of the cloaca. Furthermore, standard semen analysis involved evaluating a number of

parameters such as motility, sperm concentration, live or dead counts, and morphology of spermatozoa.

Several methods are used to assess semen quality in poultry and determine fertilizing ability. Some of these methods are regarded as highly subjective, while others require specific laboratory services (3). Progressive forward motility is a subjective but important measure of the fertilizing ability of spermatozoa. Evaluation of acrosome integrity is a reliable approach that requires specific laboratory techniques. Although the subjective methods of semen evaluation prior to its further processing or use for purposes of artificial insemination is very important, selection of breeding cocks based on semen quality is equally important (4-7).

Many factors are involved for achievement of full reproductive potential in breeding domestic chicken and red jungle fowl cocks, such as dominance status (8-11), intensity of courtship behavior (12), moment of mating, male mating interference (13), motility, and sperm concentration (14). In previous studies, the semen characteristics of some species such as drakes

* Correspondence: wahid@vet.upm.edu.my

(15), cocks (16,17), ganders (18), turkeys (19,20), and pheasants (21) were reported. Therefore, the present study was conducted to compare the ejaculate quality, including volume, concentration, percentage of motility, and types of abnormalities, in red jungle fowl, domestic chicken, and bantam chicken in Malaysia.

2. Materials and methods

2.1. Animal and semen collection

The experiment was carried out in the Theriogenology and Cytogenetic Laboratory of the Faculty of Veterinary Medicine, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). A total of 27 healthy cocks included 9 cocks each of red jungle fowl, domestic chicken, and Malaysian bantam chicken with average weights of 410 ± 2.34 , 1.225 ± 4.41 , and 1.670 ± 6.01 g, respectively. Cocks aged around 36 weeks old were used in this study. The cocks were obtained from the Department of Animal Science at the UPM Poultry Farm ($2^{\circ}6'N$, $103^{\circ}24'34''E$). The mean ambient temperature and relative humidity during the study period were $30^{\circ}C$ and 65%, respectively. One month prior to commencement of semen collection, all cocks were kept in individual cages ($32 \times 34 \times 53$ cm). All cocks were fed with commercial poultry pellets consisting of 18% crude protein and water was provided ad libitum.

Semen samples were collected once a week (Mondays) and were continued for 16 weeks. Semen collection was done once a week because the time range required for semen to pass from the testes to the distal region of the ductus deferens varies from 1 to 4 days (22). The time of semen collection was between 0800 and 0900 hours, and the method of collection was the abdominal manual massage method described previously (23,24). First, the cloacal area was cleaned. The back and tail feathers and the abdominal region were then stroked gently and repeatedly, which resulted in the tumescence (erection) of the phallus. Semen was ejaculated after slight pressure was applied to the inverted cloaca. The semen was carefully collected in a test tube and placed in a water bath maintained at $37^{\circ}C$ prior to evaluation.

2.2. Semen evaluation

Sodium citrate and egg yolk were prepared and used as an extender in this study. The volume of the ejaculated semen from each cock was measured using a graduated test tube. The pH was determined using a pH meter. Spermatozoa concentration was determined using the hemocytometer method, and the technique involved mixing semen with appropriate diluents at a dilution ratio of 1:200 with an eosin solution (1). The evaluation of sperm motility from the diluted semen was conducted at $400\times$ magnification on a warm stage. A drop of the diluted semen was placed on a preheated slide and a cover slip was used to cover the slide; the cover slip helped to prevent overflow, allowed a uniform film to form, and prevented quick drying of the semen (14). The remnant of the semen in each replicate was measured to evaluate the percentage of live and dead spermatozoa as determined from $10 \mu L$ semen, mixed with $50 \mu L$ of eosin nigrosin stain to make a thin smear. The smear was air-dried for 10 min. At least 200 spermatozoa were examined ($400\times$) under emulsion oil and those with differential morphology were counted. The color and consistency of the semen were evaluated visually, including varieties that were creamy, grainy, bloody, watery, or contaminated.

2.3 Statistical analysis

Data were analyzed using SPSS 16. Repeated measures analysis of variance (ANOVA) of the general linear model in SPSS was used for the analysis. Analyses were considered to be significant at $P < 0.05$.

3. Results

The average pH of the cocks' semen in this study was 7.0–7.4. The semen of the bantam chicken was watery. The volumes of semen and concentrations were 0.33 ± 0.16 , 0.29 ± 0.18 , and 0.10 ± 0.10 ejaculate/mL and $4.44 \times 10^9 \pm 9.05$, $2.73 \times 10^9 \pm 10.5$, and $1.83 \times 10^9 \pm 7.43$ sperm/mL for red jungle fowl, domestic chicken, and bantam chicken, respectively (Table 1). The semen volume was not significantly different ($P > 0.05$) between the red jungle fowl and the other breeds, whereas the semen

Table 1. Means of volume, concentration, percentage of motility, live spermatozoa, and total abnormality of semen red jungle fowl, domestic chicken, and bantam chicken.

Item	Breeds of cocks		
	Red jungle fowl	Domestic chicken	Bantam chicken
Volume (ejaculate/mL)	0.33 ± 0.16	0.29 ± 0.18	0.10 ± 0.10
Concentration (sperm/mL)	$4.44 \times 10^9 \pm 9.05^a$	$2.73 \times 10^9 \pm 10.5^{ab}$	$1.83 \times 10^9 \pm 7.43^b$
General motility (%)	55.2 ± 6.67	57.1 ± 4.56	49.0 ± 6.03
Live spermatozoa (%)	96.4 ± 0.86	97.5 ± 1.28	96.0 ± 2.27
Total abnormality (%)	31.4 ± 3.70	30.3 ± 6.91	38.0 ± 4.12

Values within each column with different superscripts differ significantly at $P < 0.05$ ($n = 16$).

concentrations were significantly higher ($P < 0.05$) between the red jungle fowl and bantam chicken.

The percentages of spermatozoa motility, live spermatozoa, and total abnormality of the semen were $55.2 \pm 6.67\%$, $57.1 \pm 6.67\%$, and $49.0 \pm 6.03\%$; $96.4 \pm 0.86\%$, $97.5 \pm 1.28\%$, and $96.0 \pm 2.27\%$; and $31.4 \pm 3.70\%$, $30.3 \pm 6.91\%$, and $38.0 \pm 4.12\%$ for red jungle fowl, domestic chicken, and bantam chicken, respectively. Furthermore, the percentage distribution of individual motility (forward, rotating, vibrating, and backward) are shown in the Figure. Red jungle fowl had significantly higher ($P < 0.05$) sperm motility compared to the domestic chicken and bantam chicken. All the breeds had a total live spermatozoa of more than 90%. The mean live spermatozoa among the red jungle fowl, domestic chicken, and Malaysian bantam chicken were not significantly different (Table 1).

In the present study, the percentage spermatozoa abnormalities, including mid-piece knotting, bent head,

plasmid droplets, spermatids, and bent tail were low ($<10\%$) in all the breeds (Table 2). Furthermore, the bantam chicken had significantly higher macrocephalic abnormalities than the red jungle fowl.

4. Discussion

In this study, there were variations among the breeds in the quality and quantity of spermatozoa. Generally, poultry semen is low in volume but high in concentration of spermatozoa. In the present study, the semen volume (0.10–0.33 mL) was similar to the results reported for other adult cocks of different genetic backgrounds (0.29–0.52 mL) in previous studies (25). According to Sturkie (26), the semen volume in pheasant was 0.39 mL, duck 0.3 mL, and goose 0.1–0.6 mL.

This study also determined that the semen pH was within the normal range. The pH of cock semen is 7.0–7.6, depending on the amount of transparent fluid present

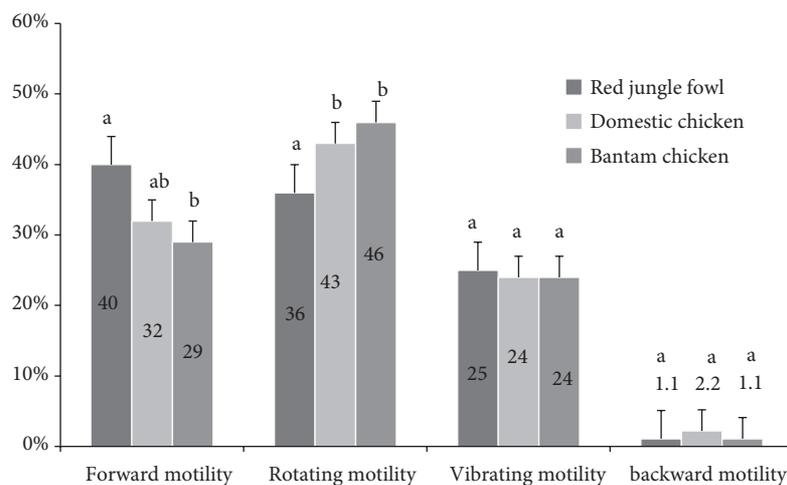


Figure. Mean \pm SD of sperm general motility and individual motility of red jungle fowl, domestic chicken, and bantam chicken. Columns with different superscripts differ significantly at $P < 0.05$ ($n = 16$).

Table 2. Means of types of spermatozoa morphological defects of red jungle fowl, domestic chicken, and bantam chicken.

Types of abnormalities	Breeds of cocks		
	Red jungle fowl (%)	Domestic chicken (%)	Bantam chicken (%)
Macrocephaly	22.3 ± 4.38^a	21.5 ± 4.97^a	27.4 ± 2.50^b
Mid-piece knotting	4.4 ± 1.79	3.9 ± 2.27	4.6 ± 1.81
Bent head	1.2 ± 1.18	2.2 ± 1.64	1.2 ± 1.39
Cytoplasmic droplet	1.2 ± 0.79	1.3 ± 1.06	2.0 ± 1.25
Spermatid	1.52 ± 0.75	0.66 ± 0.91	1.0 ± 1.06
Bent tail	1.83 ± 1.54	0.44 ± 0.68	1.78 ± 1.84

Values within each column with different superscripts differ significantly at $P < 0.05$ ($n = 16$).

(27). The watery semen color of bantam chicken might be due to a low number of spermatozoa per milliliter of semen. Similarly, the average semen concentration in the red jungle fowl and domestic chicken were within the range reported in leghorn roosters (1.7–3.5 billion/mL) in a previous study (16), but lower than that in broiler breeders (6.2×10^9 sperm/mL) reported by McDaniel (6). Semen concentration in the red jungle fowl was 4 times higher than in the bantam chicken. The differences in semen concentration between chickens is suspected to involve many factors such as intake of feed, and the body size could be attributed to their different genetic makeup and body weight.

The assessment of sperm motility is one of the most often used parameters for semen evaluation (28–30). The values obtained for semen motility for all 3 breeds were within the range reported for normal cock semen of 40%–80% (16,25,31). Howarth (32) suggested that spermatozoa collected from cock testes, despite very poor motility, were able to fertilize the ovum, because the oviduct itself ensures their movement towards infundibulum. All 3 breeds of

chicken had a live spermatozoa percentage of more than 90% and this is comparable with a previous study reported for broiler breeders (33–35).

Sperm morphology was recommended to be one of the most essential qualitative characteristics of poultry semen (36). It could be used as an essential parameter for predicting the fertilizing ability of spermatozoa (37). The analyzed sperm abnormalities in this study were macrocephaly, mid-piece knotting, cytoplasmic droplets, spermatids, bent head, and bent tail. The percentage of abnormalities that were part macrocephalic was more elevated than others, but the total abnormality rate had similar results to adult cocks of different backgrounds (22.0%–48.0%) in previous studies (25). In conclusion, the red jungle fowl has a better concentration of spermatozoa than other breeds.

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