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SEMEN AND SPERM CHARACTERISTICS OF NUNUKAN ROOSTER

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ABSTRACT

The objective of this study was to characterize the sperm and semen of nunukan rooster. Twenty roosters were examined in this study for quality characteristics. Semen was collected twice a week by dorso-abdominal massage method. Parameters evaluated were volume, concentration, motility, live/dead ratio, and abnormality percentage. Data were analyzed descriptively for each parameter. The result of this study showed that the semen was white-milk in color, spermin in the smell, and thick in consistency. The semen had an average pH of 7.2±0.1. Furthermore, the mean value measurement for volume was 0.21±0.15 mL, sperm concentration was $3.68\pm0.53x10^9$ sperm/mL, the percentage of sperm motility was 80.00±0.50 %, the percentage of live sperm was 82.55±16.51 %, and percentage of abnormal sperm was 18.20±10.40 %. The seminal plasma in every 100 mL contained 2.1 g protein, 4 mg fructose, 8 mg sorbitol, 319 mg sodium, 60 mg potassium, 8 mg calcium, 12 mg magnesium, and 138 mg chloride.

Keywords: semen, sperm, nunukan rooster

INTRODUCTION

Nunukan chicken is one of the germ-plasma livestock East Kalimantan, which has its own characteristics and advantages of the potential genetic to be preserved and developed. The characteristics of nunukan chicken, among others, males and females have brown fur as a base color, plain fur for color pattern, flickering golden fur, and smooth complexion feather. Another characteristic of nunukan chicken is the slow growth of feathers on the wings and tail, very short or even not grow at all (Sartika *et al.*, 2006). The weight of the original nunukan chicken can reach 4 to 5 kg's, 20-30% heavier than other local chickens and the egg production could reach 182 per year with the average egg mass of 47.5 grams (Wafiatiningsih *et al*., 2005).

However, in the conventional breeding that does not have a good breeding program, a number of males are very limited, unbalanced proportion if it is compared with the number of females. It can lead to the low overall productivity of the nunukan chicken. Although the contribution of male (sire) in livestock productivity was limited to the sperm, the availability that will be a regenerative material from the male form of the sperm is needed.

Assessment of semen quality based on its characteristics gives an excellent indicator of poultry productive potential. The quality of semen leads to succeeding in fertility and hatchability of eggs. However, there are many differences in semen quality depend on the strain, age, body size, nutrient feed, temperature environment as well as types of chicken (Almahdi *et al.*, 2014). So far, no comprehensive studies have been carried out to evaluate the nunukan rooster's semen so that ensuring the quality from their characteristics is needed to know their reproductive potential.

The effort in improving the productivity of the offspring can be done by Artificial Insemination (AI) program. Mating with AI method requires a number of males were much less, depending on sperm production and sperm needed by the female to maintain productivity and the development of nunukan chicken. Both male and female contribute significantly to the level of chicken fertility. Therefore, the quiet information about characteristics and quality of sperm and semen of nunukan chickens is considerably available in order to improve the productivity of nunukan chicken.

RESEARCH METHOD

Animals

This study used twenty nunukan roosters weighing 3-4 kg's per head, aged 30-35 weeks. Each rooster was placed in an individual cage, 50 x 50 x 75 cm^3. The commercial feed was given twice per day as much as 100-150 g/day which drinking water provided ad libitum. Vaccination programs, provision of deworming and vitamin conducted regularly.

Semen Collection

Semen was collected by using massage or sorting on the dorso-abdominal gently from the back to the base of the tail and cloaca until the roosters aroused and shown with a raised tail. The massage was done with a certain pressure until the white sperm discharge from the cloaca and collected using the large-scale tube. Prior to semen collection, the part around the cloaca cleaned with tissue paper that had been sprayed with physiological Sodium Chloride solution.

Semen Evaluation

Evaluation of the semen quality includes observation and measurement of the volume, pH, color, and density as well as a microscopic examination to determine the concentration, motility, viability and morphology abnormality. The volume of the semen was carried out by the scale on the tube. The degree of acidity (pH) was measured using pH indicator paper (BTB, scale 6.4-8.0) and observation of color are made directly to a shelter before the semen was inserted into the tube shelters, then density was determined at the making of semen from the tube.

In addition, microscopic observation was made after dilution. Observation of individual spermatozoa movements was performed to see the activity of the progressive movement of spermatozoa per unit. Observations were carried out by dropping the semen which had been diluted, then covered with a lid glass, and then observed under a microscope with a magnification of 40x objective lens. The observation of progressive spermatozoa movements was conducted subjectively in ten different fields of view in the range of 0-100 % with 5% interval scale.

Furthermore, sperm concentration was counted using hemocytometer with Neubauer counting chamber. The collected semen was taken using micropipette in 1: 200 in NaCl 0.9% as much as 10 µL. Later on, each 10 µL of semen was dropped on cover glass side and the semen spread out under whole cover glass side until counted chamber was filled. The calculation on the counted chamber was done on 5 boxes. The sperm concentration is the number of sperm multiplied by dilution factor and hemocytometer factor.

$$\text{Concentration} = \text{dilution factor} \times 50.000 \times \text{N cell /mL}$$

The calculation of live sperm and abnormal sperm percentage was counted by using a combination of eosin and nigrosin staining and the object was observed under a microscope with a magnification of 40x. One part of 4% eosin (4 g eosin was dissolved in 96 mL of 2.9% sodium citrate and three parts of 8% nigrosin and 8 g nigrosin dissolved in 92 mL of 2.9% sodium citrate). The indicator to determine the life sperm and dead sperm can be seen from the sperm's color. The life sperm will not absorb color while the dead will absorb the color. The percentage of abnormality was determined by counting the sperm that has abnormal forms. The calculation was performed up to 100 cells.

Analysis of Chemical Composition of the Seminal Plasma

The chemical composition of the seminal plasma which includes fructose, sorbitol, and citric acid was determined using the method of High-Performance Liquid Chromatography (HPLC), while the determination of protein, sodium, calcium, potassium, magnesium, and chloride used methods Analytical Absorption Spectrophotometry (AAS).

Data Analysis

Research conducted was an exploratory study. Data were analyzed descriptively and confirmatively with the measured variable was the average obtained from each of the variables measured. The results obtained are presented as mean ± standard deviation.

RESULT AND DISCUSSION

The dorso-abdominal massage method can be used for the collecting of semen in poultry. Semen collections of nunukan rooster take vary depending on the individual with a range of 10-30 minutes.

Characteristics of Fresh Semen

The evaluation of semen was conducted by macroscopic and microscopic observation. The macroscopic evaluation includes semen volume, color, acidity, and consistency, while the microscopic evaluation includes mass and individuals' movements, progressive motility, concentration and degree of abnormality. Generally, the characteristics of nunukan rooster semen collected shown in Table 1.

Semen Volume

The nunukan rosters' semen volume obtained at an average of 0.21 ± 0.15 mL. Generally, the semen volume of poultry is lower than mammalian semen volume, but on the other hand the sperm concentration of poultry higher than mammalian. This is in accordance with the fact that poultry does not have accessory glands,

so as not to produce seminal plasma (Mulyadi, 2007). As an indigenous rooster, the semen volume obtained was lower than other indigenous roosters. Iskandar *et al.* (2006) reported the rooster has about 0.30 ± 0.07 mL of semen, higher than Mulyadi (2007) report of 0.10 ± 0.01 mL. According to Toelihere (1993), the roosters have semen volume normally ranges between 0.3-1.5 mL.

Several factors can affect the volume of semen during semen collection which includes age, level of arousal, ejaculation frequency, and quality of feed given (Jonhson *et al.*, 2000). The laying hens weighed sexual maturity at the age of 24-26 weeks (Toelihere, 1993) and young individuals within a species produce a low semen volume. The age of the male was good to collect the semen is at the age of 1-1.5 years (48-72 weeks) (Sastrodihardjo and Resnawati, 2003) that a local chicken male age 40-80 weeks is the best semen producers. Age of the roosters used in this study ranged from 30-35 weeks so that the alleged production of spermatozoa was not optimum level yet.

Table 1. Semen characteristic of nunukan rooster

Characteristic	Average (n = 20)
Volume (mL)	0.21 ± 0.15
Color	White-creamy
Smell	Spermin
Consistency	Thick
pH	7.2 ±0.1
Motility	+++
Sperm motility (%)	80.00 ±0.50
Viability (%)	82.55 ±16.51
Dead Sperm	17.60 ±2.17
Sperm concentration ($x10^9$/mL)	3.68 ±0.53
Sperm/ejaculation ($x10^9$)	6.01 ±0.19
Normal sperm morphology (%)	80.50 ±13.40

Acidity (pH), Color and Consistency of the Semen

The pH degree obtained was 7.2 ± 0.1. This pH is lower than Isnaini (2000) report with a pH of 7.4 ± 0.2. The low degree of acidity was also suspected because of the ambient temperature around the enclosure maintenance reached up to 33-34 °C, this is according to research by Mulyadi (2007) which states that the high temperature will boost the rate of metabolism of spermatozoa in the epididymis that describes fructose in anaerobic condition so that will form lactic acid in large quantities in semen resulting in an instance of decreasing the pH degree. The low degree of acidity can also affect the viability of spermatozoa. The pH of the nunukan rooster semen ranged between 7.0-7.6 so that precautions can be considerably done is by natural mating or semen collection regularly to prevent the disintegration of fructose and the formation of lactic acid more from the metabolism of spermatozoa. The pH degree of the semen can also be affected by contamination and a number of dead spermatozoa contained in the semen as a trigger the formation of ammonia.

Based on the results of visual observation, in the nunukan chicken semen was obtained milky white with medium to thick consistency. Colors can be polluted by chicken semen mixed with feces, dirt, or blood. The consistency of semen correlated with sperm concentration. The more the number of sperm cells in semen, the more viscous consistency considering that there is no chicken semen seminal plasma.

Sperm Mass Movement

The mass movement is a movement made by a group of spermatozoa together form wave. The results showed a mass movement nunukan chicken spermatozoa relatively good (+++) is by revealing big waves, relatively thick and actively move.

Free moving spermatozoa groups in forming the wave depend on the concentration, motility and abnormality rate (Mosenene *et al.*, 2009; Pratama, 2011). The higher concentration of spermatozoa is increasingly allowing spermatozoa to move together to form waves. The process of egg fertilization in the infundibulum not just rely on one spermatozoon only, but also many spermatozoa was first in charge of opening the zone pellucid membrane vitellin with the enzyme hyaluronidase and acrosin contained in the sperm acrosome.

Motility and Individual Movement of Spermatozoa

Motility is an indicator of the ability of sperm to move properly towards an egg so that the migration of spermatozoa is also being one indication of successful fertilization. The average sperm motility obtained from this research was 80.00 ± 12:50%. The rate of sperm motility may be affected by internal and external factors. The motion of motile is caused by flagellum (tail) of spermatozoa so that their secondary abnormality (tail part) hinders the movement of spermatozoa.

Movements of individual spermatozoa correlated with the mass movement of spermatozoa were observed. According to Mulyadi (2007), motility of spermatozoa arises because individual spermatozoa moving randomly in all directions, free, without

interdependence with each other. In this study, the individual motions of spermatozoa relatively good with the average score of 2 – 4, so that the mass movement of spermatozoa shown relatively good as well (+++).

Sperm Concentration

The concentration of spermatozoa shows the number of sperm per mL of semen ejaculated. Spermatozoa of poultry are higher than the concentration of spermatozoa other livestock because the birds do not have glands in mammal accessories such as plasma producing cement, so that even though the cement produced lower but it has more sperm concentration. The average concentrations of the spermatozoa of this study were at 3.68±0.53 x 10^9 / mL, lower than the results of studies of other chickens.

Several factors affect the concentration of spermatozoa ranging from the amount of ejaculate volume, frequency of shelter, stud and environmental conditions (Prastowo, 2008). The volumes of the ejaculate produced in this study were moderate at 0.21 ± 0.15 mL, showed that the concentration of spermatozoa was ~~is~~ also lower. The frequency of semen shelters in nunukan chickens are also thought to influence the concentration of spermatozoa, because the frequency of an organized collection can increase libido, thus spurring spermatogenesis in the testes. Besides, per individual physiological conditions also need to be considered related to the level of stress due to the changes in differences in environment and reproductive conditions.

Semen Characteristic

Similar to other chickens, nunukan rooster's semen has complex compounds including protein, fructose, sorbitol, sodium, potassium, calcium, and magnesium (Table 2).

Table 2. Comparison of Characteristics of Nunukan's Rooster Semen with Local Rooster

Component	Nunukan rooster	Local rooster*)
		(mg/100mL)
Protein	2.1	1.8-2.8
Fructose	4	4
Sorbitol	8	0-10
Sodium	319	352
Potassium	60	61
Calcium	8	10
Magnesium	12	14

**) Garner & Hafez (2000)*

The seminal plasma is not required at the time of fertilization but essential for natural mating in which plasma is needed as carrier fluid spermatozoa. Apart from being a carrier (transport medium), the seminal plasma also provides nutrients (e.g. fructose and sorbitol) and protective factors (buffer) to keep fixed semen alkalis while the acidic vaginal fluids. Keeping the seminal plasma motility is important in order to improve the viability of spermatozoa and to increase the resistance of swine sperm membrane damage due to cold shock (Barrios *et al.*, 2000).

Sperm Morphology

The spermatozoa of poultry generally have parts in common with other mammals' livestock spermatozoa but different forms of their fowl. Poultry spermatozoa have a cylindrical head with the acrosome on endpoint, the middle of a short and a longer tail (Etches, 2000). The Spermatozoa of chicken have a slightly curved head that consists of acrosome and nucleus. The tail consists of the neck, mid-piece (middle) and the main part of the tail (Nuryadi, 2001). The head of rooster's spermatozoa is simpler than others because of the acrosome sac was not until the equatorial segment as well as mammalian spermatozoa (Etches, 2000).

Figure 1. Nunukan rooster's semen shows that the live spermatozoa do not absorb the red color in Eosin Nigrosin staining 100x

This study showed that the average nunukan rooster had abnormal spermatozoa at 18.20± 10.40%. The parameters in determining male fertility based on the morphology of spermatozoa can be seen from the level of its abnormality (Aditya, 2008). High spermatozoa abnormalities can affect fertility (Yudi *et al.*, 2010). Spermatozoa abnormalities caused by several factors such as disease, heat stress

management (maintenance), cryopreservation process, differences in race and strain chicken, and season. In addition, the rate of abnormalities also can be caused by post-collection preservation and staining.

The high ambient temperatures reached up to 33-34 °C is expected to affect the formation of spermatozoa in the testes, which are generally impaired spermatogenesis produce primary abnormality or abnormalities in the head. According to Gunawan and Sihombing (2004), a high temperature of about 25-31 °C can reduce the productivity of reproduction in commercial laying hens and presumed maximum limit for broilers higher based on adaptability. At the high temperatures, chicken or rooster need more energy for the regulation of body temperature, thereby reducing the availability of secondary energy for reproduction and hormonal metabolism.

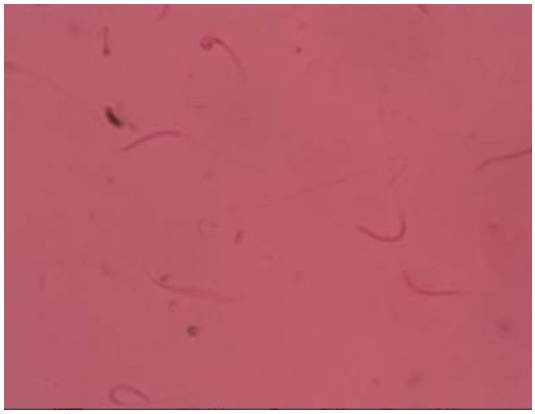

Figure 2. Some of abnormal spermatozoa forms found; spermatozoa with circular tail and the cytoplasmic droplet in proximal, head without tail, tail without head, and head and tail curve (coiled)

Abnormalities of spermatozoa are classified into two; primary and secondary abnormalities (Barth and Oko, 1989). Primary abnormality may occur due to abnormalities in the process of spermatogenesis that occurs in the tubules seminiferous. Then, secondary damage of spermatozoa abnormalities during its passage through the epididymis, during the phase of ejaculation, or after ejaculation occurs include excessive heating process, cooling fast, contamination with water, urine, and antiseptic (Yudi *et al.*, 2010). Primary abnormality occurs in the head, had a double tail, curled tails (around the head), drop out or be split into two. Meanwhile, secondary abnormalities include head without a tail, the central part of the fold, their cytoplasmic granules and a proximal or distal sheath detached acrosome (Hafez and Hafez, 2000).

CONCLUSION

The nunukan rooster semen was white-milk in color, spermin in smell, and thick in consistency with an average of pH of 7.2±0.1. Each parameter measured was the mean value for sperm volume of 0.21±0.15 mL, sperm concentration was $3.68\pm0.53x10^9$ sperm/mL, the percentage of sperm motility was 80.00±0.50 %, percentage of live sperm was 82.55±16.51 %, and percentage of abnormal sperm was 18.20±10.40 %, respectively. Meanwhile, the chemical compositions in every 100 mL of the seminal plasma contained 2.1 g protein, 4 mg fructose, 8 mg sorbitol, 319 mg sodium, 60 mg potassium, 8 mg calcium, 12 mg magnesium, and 138 mg chloride. Nunukan rooster had relatively the same values of these substance concentrations compared to other domestic roosters.

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