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Semen Quality of Sentul Cock with Different Immunoglobulin Yolk Concentrations

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ABSTRACT

The aim of this study was to evaluate the effect of different IgY concentrations on the quality of semen (macroscopic and microscopic) and testicular morphometry (weight, length and width of the testes) of Sentul cock testis and its ability to fertilize hens. This study used 4 months of 20 Sentul cocks consisting 10 high IgY concentration cocks and low IgY concentration cocks. This study used a complete randomized design (RAL) with 2 treatments namely low and high IgY concentration and 10 times repetitions. Data were analyzed using t-test minitab program versions 16. Based on the result, different IgY concentrations did not affect the quality of semen and testicular morphometrics, but high IgY concentration cocks were able to fertilize more hens than the low IgY concentrations one by using dilution methods. This proves that high IgY concentration cocks are more effective and efficient cocks or stud.

Keywords: IgY, Morphometric Testis, Semen quality, Sentul cocks

Introduction

Indonesia has 31 local chicken families; one of them is Sentul cock (Nataamidjaya, 2010). Sentul cock is one of the Germplasm originating from Ciamis District, West Java. This cock is very potential to be commercially bred to fulfill people's nutrition and increase farmers' income (Sulandari *et al*. 2007). Muhsinin *et al*. (2016) stated that Sentul cock was able to neutralize S. pullorum bacteria by 26% -60% through in vitro testing that using the clearance test method with a dose of 107 CFU mL-1. This situation proves that the resistance of Sentul cocks varies greatly. IgY is a protein molecule substance is able neutralize a number of microorganisms that cause infection. IgY can be found in blood serum and egg yolk (Carlander, 2002; Raj *et al*., 2004). Total IgY in chicken blood serum is 5-15 mg mL-1 and total IgY in egg yolk ranges from 10-25 mg mL-1 (Gaetani *et al*., 2017).

High resistance animal can produce a good performance because the immune system is supported by immune cell function and antibody production (Regar *et al*., 2013). The better the defense system, the more resilient the body's immune system against various infectious agents. Chicken body resilience can be obtained actively and passively. Vaccination is one way to actively gain endurance, while maternal immunity is a way to obtain passively endurance (Ulupi, 2014).

Conservation and development of local chickens can be reached by improving the quality of the seeds produced. Cock has a very big role in producing offspring and improving the performance of the next generation. The high IgY cock is a more resistant and healthier cock from disease especially in the metabolic process. Reproductive organs with these conditions are expected to produce quality semen therefore the information about concentration of IgY in the metabolic process is important to know, thus the purpose of this study is to evaluate the quality of semen (macroscopic, microscopic and the ability of Sentul cocks to fertilize hens). Sentulcocks that have different IgY concentrations in the blood serum.

Materials and Methods

Cocks Research

The cocks used were 20 Sentul cocks, 4 months old. Cocks body weight at the start of the study was around 1.6-2.2 kg bird^{-1} with an average of 1.9 kg bird^{-1}. The cocks is the result of selection from the Animal Breeding and Genetics Division of the Faculty of Animal Science, Bogor Agricultural University.

Testing the IgY concentration of Sentul cocks

Testing of IgY concentrations was carried out on 20 Sentul cocks. Testing of total IgY in blood serum was carried out using the indirect

method of ELISA (Enzyme-Linked Immunosorbent Assay) according to Yokoi et al. (2002). Cocks that have IgY concentrations above the average are classified as cock with high IgY concentrations. Cocks that have an IgY concentration equal to or below the average are classified as cock with low IgY concentrations.

Maintenance of cocks

Sentul cocks are placed in open cages in individual plots with a size of 40x60x60 cm^3. Lighting uses 2 lamps each with a power of 60 watts. The lighting process is carried out for 12 hours (18.00-06.00 WIB). Sentul cocks were maintained during the pre-layer and layer to determine their production performance. The feed used was commercial phase layer crumble from PT. Gold Coin Indonesian (protein 16-18% and metabolic energy 2,700-2,800 kcal kg^{-1}). The provision of feed and drinking water was carried out adlibitum on the morning at 07.00 WIB and in the afternoon at 16.00 WIB. The process of feeding in the morning begins with cleaning the feedlot first, then the weighed feed is added. Feeding in the afternoon is done by adding feed to the feed by following the amount that has been determined. Weighing chicken body weight is done once a week using a scale (Electronic Kitchen Scale, China). Cocks semen collection is carried out at 26 weeks maintenance.

Taking and observing semen quality

The study was conducted in the field laboratory of the Animal Breeding and Genetics Division of IPB. Semen storage begins with cleaning around the cloaca using tissues that have been moistened with NaCl. Taking cocks semen is done once for each cocks at 26 weeks maintenance age ie the early age of cock entering adulthood. Observation of semen begins by conducting a collection of fresh semen by sorting method which is done by massaging the backs of cocks to the base of the tail with the fingers of the hands with the fingers right, then continue up to get the tail. The palm of the collector's hand forms an angle of 30-40° from the back of a cock. The touch must be smooth and precise so that the cock is stimulated so that the tail is raised, the legs are slightly stretched, the cloaca opens and a pair of papillae (phallus) stand out. A Hand quickly fixes, grasps and slightly lifts the base of the tail, middle finger and thumb to press the base of the cloaca and keeps holding the two papillae stick out (Arifiantini, 2012). The semen is evaluated macroscopic and microscopic in the unit laboratory of Reproduction Rehabilitation and laboratory medical immunology of Faculty Veterinary Medicine IPB.

Macroscopic evaluation is carried out on volume, color, consistency and pH. Microscopic evaluation is carried out on mass movement sperm, motility, viability, concentration sperm and abnormality sperm.

The volume of semen is measured using a measuring pipette, the pH of semen is measured using a special pH indicator paper (Merck scale 6.4-8). The semen is dropped 5 µL on pH paper and left for 15-30 seconds then the pH paper is matched according to the standard. Observation of semen consistency is distinguished between liquid, thick and moderate. The color is divided into beige and milky white seen visually. The microscopic evaluation consists of motility, mass movement sperm, morphology, viability, concentration and number of spermatozoa per ejaculate.

Observation of the mass movement sperm is done by dripping 20 µL of semen on the glass object and then observed under a light microscope (Olympus CH 20) with a magnification of 100 times. The assessment is done by looking at sperm waves and is assessed by (+++) if the mass waves are thick and move quickly, (++) if the mass waves are thick but slow to move or medium mass waves but move quickly, (+) if the mass waves thin and slow to move and (-) if there is no mass wave (Arifiantini, 2012).

Observation of sperm motility was carried out by dropping 5 µL of semen on the glass object then dropping 10 drops of physiological NaCl. The solution is homogenized and covered with a glass cover. The preparation is observed under a microscope with magnification 400 times. Sperm motility is estimated by estimating from 5-10 field of view by comparing the number of sperm moving forward with other sperm movements, then the value is expressed in percent.

Observation of sperm viability was carried out by dropping 5 µL of semen plus 4-5 negrosin eosin solution on the glass object. The semen mixture is homogenized and then made into a pillowslip and dried over a 10-15 second heating table. Curing preparations were observed under a microscope with magnification 400 times. The percentage of live spermatozoa counted 10 fields of view with a minimum number of cells >200 cells. Live sperm do not absorb color, whereas dead sperm will absorb red color on the head. The percentage of live sperm is calculated by the formula the number of live sperm divided by the total number of sperm then multiplied by 100%.

Observation of sperm abnormality is done by using the same coloring for the examination of live sperm. Percentage of abnormality and normal sperm is performed in 10 visual fields with a minimum cell count >200 cells. The percentage of abnormal sperm is calculated by the formula the number of abnormal sperm divided by the total number of sperm and then multiplied by 100%.

The concentration of sperm was calculated using a Neubauer chamber. The semen was diluted with formol saline 500 times (2 µL of cement in 998 µL formol saline). The Neubauer chamber counting chamber was observed under a microscope with 400 times magnification. Calculation of sperm was done in 5 count rooms according to diagonal directions. Calculation of sperm concentration is based on the following formula: number of sperm calculated x25x106.

Calculation of sperm concentration based on the formula: N x 5 x FP x 106.
Information:
N : Average number of sperm in the chamber
FP : Dilution factor
5 : Correction factor, i.e. there are five boxes that count 25 boxes
10^6 : The correction factor is needed because of the depth of the slip cover Neubauerchamber of 1 ml/Neubauer chamber.

Total sperm per ejaculate are calculated by multiplying the concentration of sperm and semen volume. Data on macroscopic evaluation of semen which includes color, consistency and mass movement will be assessed with a score of 1-3 (Arifiantini, 2012). Descriptions of the assessment scores are presented in Table 1, the higher the score means the better the macroscopic semen quality.

Calculation of the ability of cocks to fertilize hens

The ability of cocks to fertilize hens by artificial insemination method, is calculated based on the multiplication of the factors of volume, motility and concentration of sperm and divided by the dose of artificial insemination (IB) which is equal to 50 million sperm/tail (Toelihere, 1993).

Observation of testicular morphometrics

Observation of the testicles begins with the slaughter of cocks Sentul and then the surgical stage is performed. White testicular organisms located in the anterior left and right kidneys are taken using scissors and continued with measurement. and the width of the testes is carried out using a caliper. Measuring the weight of the testes is carried out by weighing the testicles using a digital scale (max scale 200 g) with an accuracy of 0.1 mg. The length, width and weight of the two testes are flattened from each cock.

Data analysis

The design was used a completely randomized design (CRD) with two treatments. The treatment is the different serum (high and low) IgY concentrations. Each treatment was repeated 10 times. Each treatment was repeated ten times. Each replication consists of 1 Sentul cocks. The variables observed were semen quality. The data obtained were analyzed by t-test using Minitab program (Mattjik & Sumertajaya 2013) with the following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

Information:
n1: number of observations of meat quality with IgY levels above 9.30 ± 0.45 mg mL^{-1}
n2: number of observations of meat quality with IgY levels below 9.30 ± 0.45 mg mL^{-1}
x1: average meat sample with IgY level above 9.30 ± 0.45 mg mL^{-1}
x2: average meat sample with IgY level below 9.30 ± 0.45 mg mL^{-1}
s1: standard deviation of meat with IgY level above 9.30 ± 0.45 mg mL^{-1}
s2: standard deviation of meat with IgY levels below 9.30 ± 0.45 mg mL^{-1}.

Result and Discussion

Quality of semen

The results of observing the characteristics of Sentul cocks semen macroscopically include volume, pH, consistency, color (Table 2) and microscopically which include sperm mass movements, sperm motility, sperm viability, sperm concentration, sperm abnormality (Table 3).

The results of observations of semen quality macroscopic and microscopic did not show any statistical difference. This was since during the process of semen collection and semen evaluation, cocks Sentul during the period of this layer were in good health. These conditions indicate that all organs function properly, as well as their reproductive organs, so that all cocks produce semen with no different quality.

Sentul cocks that have low IgY concentrations with values below 9.30 ± 0.45 mg mL-1 produce lower semen volume (0.09 mL) compared to the study of Junaedi et al. (2016) which is equal to 0.13 mL. This study used cocks

Table 1. Description of evaluation score of macroscopic semen

Score	Evaluation Macroscopic Semen		
	Colour	Consistency	Movement Mass
1	Cream	Liquid	+
2	Milk white	Medium	++
3	Cloudy white	Thick	+++

Table 2. The macroscopic characteristics of Sentul cocks semen with different IgY concentrations

Variables	Low IgY	High IgY	Reference*
Macroscopic			
Volume (mL)	0.09±0.02	0.14±0.03	0.13
pH	6.74±0.04	6.73±0.11	7.07
Colour	1.86±0.14	1.71±0.18	Milk white
Consistency	2.71±0.18	2.86±0.14	Thick

*Junaedi *et al*. (2016).

Table 3. The microscopic characteristics of Sentul cocks semen with different IgY concentrations

Variables	Low IgY	High IgY	Reference*
Microscopic			
Movement mass sperm	1.86±0.26	2.14±0.26	3.00
Motility (%)	68.00±3.40	70.00±4.20	83.33
Viability (%)	84.37±1.50	81.72±2.20	92.36
Concentration sperm (x10^9cell mL^{-1})	2.16±0.51	3.35±1.10	2.96
Abnormality sperm (%)	6.79±1.20	6.82±1.50	7.05

*Junaedi *et al*. (2016).

Table 4. The ability of Sentul cocks with different IgY concentrations in hens fertilize

Variables	Low IgY	High IgY
Volume (mL)	0.09±0.02	0.14±0.03
Concentration sperm (x10^9cell mL)	2.16±0.51	3.35±1.10
Concentration sperm/ejaculate (x10^9cell mL)	0.19±0.02	0.47±0.02
Motility (%)	68.00±3.40	70.00±4.20
Motility Sperm/ejaculate (million)	129.20	329.00
Dose sperm IB/tail (million)	50.00	50.00
The ability in hens fertilize (cocks)	2.29	6.29

Table 5. Testicular morphometry of Sentul cocks with different IgY concentrations

Testicular morphometrics	Low IgY	High IgY
Weight (g)	6.42±1.30	6.86±1.40
Long (cm)	3.36±2.50	3.37±2.40
Wide (cm)	1.67±1.20	1.67±1.40

Table 6. Performance of prelayer and layer phases Sentul cocks during the research

Variables	Low IgY	High IgY
Prelayer		
Feed consumption (g $chicken^{-1}$ day^{-1})	123.60 ± 1.83	122.87 ± 3.38
Daily weight gain (g $chicken^{-1}$ day^{-1})	14.30 ± 2.59	15.98 ± 3.86
Feed Conversion	9.08 ± 1.73	8.36 ± 1.15
Morbidity (%)	30	0
Mortality (%)	0	0
Layer		
Feed consumption (g $chicken^{-1}$ day^{-1})	122.97 ± 0.47	123.00± 3.38
Daily weight gain (g $chicken^{-1}$ day^{-1})	11.34 ± 1.94	10.98 ± 1.80
Feed Conversion	11.88 ± 3.12	11.57 ± 1.95
Morbidity (%)	0	0
Mortality (%)	0	0

around 6 months old and in the study of Junaedi *et al*. (2016) Sentul cocks used were 12 months old. The age of 6 months in cocks is the initial age of entering the layer period.

The volume of semen produced in this study is still low. This is by following Hijriyanto *et al*. (2017) which states that older cocks can produce more semen volume.

The microscopic quality of sperm concentration also shows the same value as the volume of semen. Sentul cocks with low IgY concentrations produce lower sperm concentrations than the results of the study of Junaedi *et al*., (2016), but in cocks with high IgY concentrations of sperm produced higher than the results of these studies.

The ability to fertilize a hen

Reproductive performance of Sentul cocks with different IgY can not only be seen based on the partial quality of semen. The potential of Sentul cocks can also be seen based on the ability to fertilize the hen. Calculation of the ability of Sentul cocks to fertilize a hen can be done by multiplying three factors of semen quality which are very important namely semen volume, sperm concentration and motility of sperm. These three factors are multiplier factors in diluting semen. Dosage sperm for IB in hen are 50 million per head (Toelihere, 1993). Based on the above description calculations to determine the ability of Sentul cocks to fertilize hens by the IB method is presented in Table 4.

The results showed that a Sentul cocks with high IgY was able to fertilize about 6 hens for each ejaculation and a Sentul cocks with low IgY could only fertilize about 2 hens for each ejaculation. The results of this study indicate that Sentul cocks that have high IgY concentrations are more efficiently used as cocks because they can fertilize more hens.

Morphometric testes

Testicular morphometry was measured in layer period cocks, namely the weight, length and width of the testes. Morphometric analysis of Sentulcocks testes that have different IgY concentrations the results of the study are presented in Table 5.

Weight, length and width of the test do not indicate any difference. This is caused by the difference in body weight and volume of semen produced by Sentul cocks with different IgY concentrations (Table 6). According to Okpe et al. (2010); Hahn *et al*. (1969); Lubis and Winugroho (1984) stated that between testes, body weight and spermatozoa production influence each other.

Conclusions

Different IgY concentrations did not affect semen quality and morphometry of Sentul cocks testes. Cocks that have high IgY concentrations can fertilize more hens than low IgY so that Sentul cocks with high IgY concentrations are more effective and efficient to use as cocks.

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