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The Quality of Kampung Broiler (KB) Chicken Frozen Semen with DMA Concentrations on Yolk Lactate Ringer Diluent

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Abstract

Chicken Kampung Broiler (KB) is the result of a cross between a local chicken (chicken) with broiler imported chicken (broiler chicken). Testing the quality of frozen chicken semen from crossing needs to be done to get high productivity. This study aims to obtain a concentration of cryoprotectant *Dimethyl Acetamide* (DMA) the best of the concentration of 5%, 7% and 9% in chicken semen freezing KB. The semen used in this study came from three chickens of KB and repeated as many as nine times. Semen is collected by the method of message three times a week. Semen is evaluated macroscopically and microscopically. Subsequently spermatozoa were diluted with yolk diluent and the addition of three DMA cryoprotectant concentrations (5%, 7%, and 9%). Semen diluted 0:25 ml is packed into *straw.* Then equilibrated at a temperature of 5 ˚C for two hours. After equilibration evaluation of motility and viability of spermatozoa. It is then frozen on liquid nitrogen vapor for 10 minutes. Frozen semen is then stored in liquid nitrogen containers at a temperature of -196 ˚C. After 24 hours, the *thawing* semen in 37 ˚C for 30 seconds. The results showed that the addition of DMA 9% in Ringer's lactate diluent yolk produces a percentage motility and viability after *thawing* is greater than 5% and DMA 7%. Neither the addition of DMA 9% concentration can increase *recovery rate* after *thawing.*

Keywords: frozen semen, chicken KB, DMA

A. Introduction

Chicken Kampung Broiler (KB) is the result of a cross between a local chicken (chicken) with broiler imported chicken (broiler chicken). A cross between a broiler chicken with opportunities to perform these types of livestock breeding, so it can produce a new race or

nation chicken *(proven breed)* whose productivity is better. Testing the quality of frozen chicken semen from crossing needs to be done to get high productivity. Frozen semen that has poor quality causes low fertility of eggs and vice versa for good quality frozen semen will result in better percentage of fertile eggs.

Semen freezing is an effort used to prolong the life-force of spermatozoa. The cryopreservation technique will be an invaluable tool for the poultry industry (Fulton, 2006) and for the continued success of the remaining genetic resources of poultry. But in the freezing process frequently encountered is *cold shock* and cell damage caused by the formation of ice crystals. Processing of frozen semen needs to pay attention to several factors such as dilution and precise cryoprotectant concentration so as to protect the spermatozoa from clotting effect. Semen diluent should contain a source of nutrients, buffers, preventing *cold shock,* cryoprotectant, and antibiotics.

Semen freezing needs to be added a protective agent commonly referred to as cryoprotectants that can prevent ice crystals from forming and stabilize the plasma membrane of spermatozoa during clotting process. The cryoprotectants used should be readily soluble in water and should have small molecular weights for faster penetration into cells and reduce toxicity due to high osmolarity. Proper cryoprotectant concentrations in the diluent are essential for protecting the spermatozoa during freezing (Suidzinska & Lukaszewicz, 2008).

Cryoprotectant grouped into cryoprotectant who work inside and outside the cells (Chen, Lien, Chen, Chang, Tsai, & Yang, 2005; and Luz, Holand, Pereira, Teixeira, Vantini, & Freitas, 2009). *Dimethyl acetamide* (DMA) is a type of cryoprotectant that working in the cell, molecular weight 87.12 g / mol and a specific gravity of 0.94 g / cm ^{3} is a solution that is easily soluble in water, alcohol, ether, acetone, benzene and other solvent. DMA cryoprotectant have good penetration in the cells with membrane *lipid* content of the lot. DMA optimum dose in semen thinners ducks by 10% (Han, Niu, Liu, & Yang, 2005), and Arab chicken by 7% (Iskandar, Mardalestari, Hernawati, Mardiah, & Wahyu, 2006).

In addition to the type of cryoprotectant used, the concentration of cryoprotectants will also affect the quality of frozen semen. The diversity of poultry spermatozoa quality as well as the optimum effectiveness of cryoprotectants in cryopreservation process are different from the results of each researcher. Earlier research got optimum performance from DMA that is 5-10%. This study was conducted to determine the effectiveness of each concentration of DMA cryoprotectants (5%, 7% and 9%) in the yolk lactate diluent yeast on freeze semen of KB cocks.

B. Methodology

1. Sources of Semen

Rooster used in this research is 3 chickens of crosses and kampung broiler (KB). Rooster used is a sex and 1 year old. Rooster is individualized. Rooster used is adapted to semen collectors and in cage environments. Adaptation takes two months. Once accustomed to the environment of cages and collectors, new chickens can be used in research to accommodate the semen.

Feed given in the form of feed so (commercial food) in the form of pellets from the company PT. Gold Coin Indonesia. Feed is given at a dose of 150 grams / day and drinking water in ad libitum. The nutrient content of feed can be seen in Table 1.

Table 1 Nutrient content of feed from PT. Gold Coin Indonesia.

Composition	Nutrient content
Crude protein (%)	17 .00
Ash (%)	14 .00
Crude fibre (%)	6 .00
Rough fat (%)	3 .00
Calcium (%)	4.2 0
phosphor (%)	0.60

2. Materials and Equipment

The materials used for research include physiological Na-Cl, egg yolks, ringer lactate, antibiotics (penicillin and streptomycin), dye eosin negrosine, alcohol, paper towels, liquid nitrogen, and cryoprotectant *dimethyl acetamide* (DMA) at a concentration of 5%, 7 %, And 9%.

Equipment used for the storage and evaluation is the syringe 1 ml, 2 ml *microtube,* microscopes, glass objects, glass coverings, shelf semen tube, *counting chamber,* plastic pipette, scissors or *straw cutter,* and *refrigerators.* While the equipment involved to cryopreservation semen including the freezing and thawing of frozen semen which include collection tubes semen 2 *ml,* test tubes 5 ml, micropipette, tip micropipette, *sterofoam* box, set the storage of frozen semen *(storage container)* or container of liquid nitrogen (-196 ^{o} C), pipette, plastic straw *(straw)* measuring 0:25 ml, 1 ml syringe fitted with connectors straw, tube racks, semen in a plastic box, Bunsen, thermometer, and a refrigerator.

This research was carried out in Poultry Breeding Cage, Faculty of Animal Science IPB and Laboratory of Animal Reproduction, Faculty of Veterinary Medicine of IPB. The study was designed using a Completely Randomized Design (RAL) with three DMA cryoprotectant treatments (5%, 7%, and 9%) and nine Deuteronomy.

3. Preparation of Thinning Material

Diluents used are glucose, egg yolk, penicillin, streptomycin, DMA, and lactated ringer. DMA concentration used as diluent (Table 2).

Table 2. Composition of the frozen semen thinners used

Composition	DMA		
	5%	**7%**	**9%**
Ringer lactate (mL)	8.5	8.3	8.1
Egg yolks (mL)	1	1	1
DMA (mL)	0.5	0.7	0.9
Penicillin (IU mL $^{-1)}$	1000	1000	1000
Streptomycin (mg ml $^{-1)}$	1	1	1
Total (mL)	10	10	10
The osmotic pressure (mOsmol kg $^{-1)}$ [a]	889	1104	1294
pH [b]	7	7	7

[a] diluent osmotic pressure was measured using the osmometer. [b] pH *just* diluent (fitted) with *Tris hydroxymethyl aminomethane.*

4. Fresh Semen Collection of Chicken KB

Collection of semen chicken KB from each treatment is done three times a week. The semen shelter technique used is by sorting (massage) on the back of the chicken. Semen shelter is done by two people. One holds the chick on both of his thighs with his left hand while massage the back of the chicken to stimulate the semen out, and another prepares a scaled semen container.

5. Evaluation of Fresh Semen Chicken KB

Evaluation of fresh semen was done to find out the quantity, quality and characteristics of fresh semen from male KB chickens. Semen checks are carried out according to standard methods which include macroscopic and microscopic evaluation.

a) Macroscopic Evaluation

A macroscopic evaluation was conducted to estimate the invisible semen quality which includes; Volume, color, consistency / degree of viscosity, pH point.

b) Microscopic Evaluation

1. Spermatozoa movement includes mass movement and progressive motion Individual/motility.
2. Percentage of Spermatozoa (number of spermatozoa / ml semen
3. Percentage of Life (Viability)
4. Spermatozoa Abnormalities
5. Measurement of osmotic pressure of fresh semen and diluent

6. Semen Packing

The eligible semen is then processed. These conditions are more than 60% motility, mass movement ++ / +++, live percentage of 65% minimum. Semen diluted cryoprotectant treatment of this type of DMA at a concentration of 5%, 7% and 9%, incorporated into the *straw.*

7. Equilibrium of KB Chicken Semen

Furthermore *straw* that has been filled with semen placed in the refrigerator for equilibration process, at a temperature of 5˚C for 2 hours. Then half the semen that has been equilibrated observation of motility and viability (percentage of live spermatozoa) to determine the decline in the quality of semen after equilibration. The rest of the *Straw* that has been equilibrated then placed on a shelf freezing, placed 5 cm above the surface of liquid nitrogen at a temperature of -110 ˚ C for 10 minutes.

8. Storage

Frozen semen is stored in liquid nitrogen for further testing.

9. Frozen Chicken Semen Quality Testing Post-thawing KB

Testing is done 24 hours after storage. Di- frozen semen *thawing* in warm water (37˚C) for 30 seconds. The parameters measured in the semen inspection are the percentage of motility and viability.

The success of freezing is also assessed *Recovery Rate* (RR) is the percentage of spermatozoa were successfully recovered from the freezing process which is calculated by comparing the percentage of motile spermatozoa in fresh semen, and after *thawing.*

$$RR = \frac{Persentase\ spermatozoa\ motil\ setelah\ thawing}{Persentase\ spermatozoa\ motil\ semen\ segar} \text{ X } 100$$

C. Result and Discussion

1. Characteristic of Fresh Semen Chicken KB

Macroscopic showed good quality of fresh chicken semen (Table 3). The degree of acidity (pH) is neutral with a mean of 7.31 ± 0.06, is white, consistency is thick, mass movement is quite (++) and smells typical. The mean volume of chicken KB obtained only 0:13 ± 0:01 ml or lower than 0.2 ml volume Kampung chicken (Junaidi, Arifiantini, Sumantri, & Gunawan, 2016[a]); 0.22 ml Pelung chicken (Junaedi, 2015); and 0.17 ml Merawang chicken (Sartika, 2010). Cruciferous village chicken semen volume equal to the volume of Sentul semen broiler chickens is 0:13 ml/ejaculation (Junaidi, Arifiantini, Sumantri, 2016[b]), chicken KB low volume of research carried out due to frequent semen shelters. It shows that the frequent semen chicken shelter can cause the decrease of semen/ejaculation volume.

Table 3. Characteristics of Fresh Semen Chicken KB

Parameter	Results
Color	White
Viscosity / consistency	Thick
Mass Movement	++
Smell	Typical
PH	7:31 ± 0:06
Volume (ml / ejaculation)	0:13 ± 0:01
Motility (%)	76.66 ± 1.66
Viability (%)	88.12 ± 1:24
Concentration (Million / ml)	2623 ± 51.1
Concentration / Ejaculation (million / ejaculate)	361.04 ± 45
Total Abnormality (%)	13:44 ± 0.70
Primary Abnormalities	4:41 ± 1:01
Secondary Abnormalities	9:03 ± 0.63
The osmotic pressure (mOsmol / kg)	255

The motility of spermatozoa in chicken KB is 76.66 ± 1.66%. This figure is within the range of normal motility which ranges from > 70% according to the instructions Dumpala, Parker, & Danie, (2006). The motility of spermatozoa is an indicator of the size of the ability to fertilize ovum spermatozoa in the process of fertilization. Progressive sperm motility (move forward) become an absolute benchmark calculated. This means moving spermatozoa spin or move in place let alone that does not move is not used as a measure of sperm quality assessment. Viability of fresh semen cock KB 88.12 ± 1:24% larger than the chicken

viability *Parent stock* 82.3 ± 5.9%, based on the research results Castillo, Romboli, AIand, & Marzoni (2010) but lower than the Pelung chicken 90.97% (Junaidi, 2015) and Kampung chicken 91.14% by Junaidi, *et al.* (2016[a]).

KB chicken sperm concentration obtained was 2623 ± 51.1 million / ml, this figure is lower than some other types of chicken is 3:12 Kampung chicken billion (Junaidi, et al., 2016[a]); 2.95 billion chicken Sentul (Junaidi, et al., 2016[b]); 3.1 billion Arab chickens (Ervandi, 2011); Pelung chicken 3.19 billion (Junaedi, 2015) and 3 billion in chicken. The value of spermatozoa / ejaculation concentration Chicken KB is 361.04 ± 45 million / ejaculation.

Percentage of total spermatozoa chicken abnormalities of KB amounted to 13.44 ± 0.70%. There are two kinds of spermatozoa abnormalities found in chickens of the KB namely primary abnormalities 4.41 ± 1.01% and secondary abnormalities 9.03 ± 0.63%. In general, the abnormalities of chicken KB spermatozoa both in total abnormalities and in secondary and secondary abnormalities are high. High abnormalities affect the ability of chicken fertility.

2. *Quality of Frozen Cubed Chicken of Kampung Broiler (KB***)** *with DMA Concentration on Ringer Lactate and Yellow Egg Dilution*

The results showed that the addition of DMA to the yolk lactate ringer diluent did not affect the motility and viability of the spermatozoa after equilibration. In accordance with the results of statistical analysis showing that DMA was not significant on percentage of motility and viability of spermatozoa after equilibrium (Table 4). This is because at the time of equilibrium there has not been a drastic temperature drop (from body temperature to very low temperature) so that the effect of DMA protection has not been seen.

Table 4. Quality of frozen FP semen at various clotting stages in yellow lactate ringer diluent at various doses of DMA

Clotting stages	DMA concentration (%) [a]		
	5	7	9
Fresh semen			
Motility (%)	76.66 ± 1.66	76.66 ± 1.66	76.66 ± 1.66
Viability (%)	88.12 ± 1:24	88.12 ± 1:24	88.12 ± 1:24
After equilibration			
Motility (%)	51.11 ± 2.73	52.77 ± 1:21	56.11 ± 2:16
Viability (%)	76.25 ± 0.71	75.50 ± 1:00	76.93 ± 0:47
after *thawing*			
Motility (%)	14:25 ± 2.13b	16:29 ± 2.91b	24.44 ± 1.66a
Viability (%)	26.13 ± 2.12A	28.41 ± 2.85B	36.52 ± 1.62A
Recovery rate (RR)	18.97 ± 3.23b	21:35 ± .07ab	31.58 ± 2.31a

[a]different letters that follow the numbers on the same row and column shows the difference (Uppercase highly significant (P<0.01) in small letters while showing results significantly different (P <0.05).

Frozen semen motility after *thawing* with the addition of DMA show a significantly different effect (P <0.05). Extra DMA 9% in Ringer's lactate diluent yolk produces a percentage motility after *thawing* (24.44 ± 1.66%) greater than 5% DMA (14:25 ± 2:13%) and DMA 7% (16:29 ± 2.91%). Viability after *thawing* using DMA 9% (36.52 ± 1.62%) was higher (P<0.01) in DMA than 5% (26.13 ± 2.12% of) and DMA 7% (28.41 ± 2.85%).

The high percentage of motility and viability of sperm after *thawing* on the addition of 9% DMA capable of providing protection viability of spermatozoa during the freezing process. *Recovery rate* (RR) of semen with diluent DMA 9% (2:31 ± 31.58%) was higher (P<0.05) compared to 5% (3:23 ± 18.97%) and DMA 7% (21:35 ± 4:07%). So it can be concluded that the freeze spermatozoa using DMA diluent 9% more spermatozoa that successfully recovered after the freezing process.

The osmotic pressure test showed that fresh semen in the KB chickens had a value of 255 mOsmol / kg (Table 3). The result of osmotic pressure testing on semen diluent was 889-1294 mOsmol / kg (Table 2) showed a fairly high osmotic pressure compared to semen osmotic pressure. The result of osmotic pressure test of diluent with addition of DMA got frozen semen dissolution of chicken KB with ideal osmotic pressure that is 1294 mOmol/kg. The ideal osmotic pressure was obtained from the addition of 9% DMA concentration to the frozen semen thinners. This is consistent with the statement Junaidi (2015) that the ideal osmotic

pressure on chicken frozen semen diluents namely 1010-1230 mOmol / kg. Based on these results it is seen that the diluent has a hyperosmotic pressure. The high osmotic pressure outside the cell will cause secrete water from inside the cell, causing the cell to contract and the fluid that comes out cells replaced by cryoprotectant (Souhoka, Matatula, Mesang-Nalley, & Rizal, 2009).

D. Conclusion

Extra DMA 9% in Ringer's lactate diluent yolk produces a percentage motility and viability after *thawing* is greater than 5% and DMA DMA 7%. Neither the addition of DMA 9% concentration can increase *recovery rate* after *thawing.*

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