



Use of Old Coconut Water with Various Skim Concentrations of Milk as a Diluent for Kampung Chicken Semen

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Abstract

This study aims to determine the level of skim milk in the best coconut water that can maintain the quality of spermatozoa of native chickens during storage at 5°C. Semen was divided into five tubes (without diluents, diluents with skim milk levels 0%, 6%, 8% and 12, liquid Semen was then stored at 5°C. Observations of motility and viability were carried out at 0, 12, 24 hours, 36, and 48 hours. The results showed that the use of various diluents had a very significant effect ($P < 0.01$) on the motility and viability of chicken spermatozoa at 12 hours of storage. The highest motility of spermatozoa was found in coconut milk diluent treatment with milk level skim 6%, and skim milk level 9% at 0 o'clock, 12 o'clock and 48 o'clock storage, while the highest motility at 24 o'clock and 36 o'clock was at treatment with skim milk level 6% Viability of spermatozoa at 0 o'clock does not differ between treatments but during storage, in general, the viability of spermatozoa can survive better in diluents with skim milk levels of 6% and 9% Conclusion of this study is coconut water Diluent with skim milk level of 6% and 9% can maintain the quality of chicken spermatozoa better during storage 48 hours at a temperature of 5°C.

Keywords: skim milk, coconut water, Semen, native chicken

A. Introduction

The improvement of the genetic quality of chicken village needs to be done as germplasm owned by Indonesian people. Kampung chickens have long adapted to the Indonesian region and are relatively more resistant to the disease when compared to chicken races. Until now, the village chickens are more widely maintained using indulgence so that the control of marriage is difficult to do. Maintainance the village chickens in an individual cage as well as the use of artificial insemination technology (IB) is the right way to control the marriage to improve the

quality of its genetic. Also, the IB can increase the ratio of matrimony to streamline the maintenance of males.

Semen storage at low temperatures is one of the stages that can be done before the IB if it does not allow the implementation of the IB directly after Semen shelter. As long as the storage of Semen is important to maintain the quality of Semen to the implementation of the IB, therefore it takes the presence of Semen Diluent containing substances that are needed spermatozoa during storage. Coconut water is one of the diluents that were cheap and easy to obtain, coconut water has been used earlier researchers as a Semen Diluent such as on thin tail sheep (Kewilaa, Ondho, & Setiatin, 2013) and lamb Garut (Dwitarizki, Ismaya, & Asmarawati, 2015) with Combining that Diluent with egg yolks. According to the research results of Baba & Qistina (2015), The use of old coconut water is better used as a chicken Semen Diluent when compared to young coconut water.

Milk is an isotonic medium that contains several beneficial components to preserve the viability of spermatozoa and is used extensively by earlier researchers for cow Semen dilution (Feradis, 2010). Skim milk has a lower fat content so it can persist if it is stored in cold conditions with low humidity (Hoppe, Andersen, Jarobsen, Molgaard, Friis, Sangild, & Michaelsen, 2008). The use of skim milk in diluent has also been carried out on the Simmental cattle Semen (Yatusholikhah, Isnaini, & Ihsan, 2015), horse Semen (Azizah and Arifiantini, 2009) and Semen Entok (Hernawati, Fevianita, Hariadi, & Kurnijasanti, 2010)

Research on the use of a combination of coconut water and skim milk has never been done on local Indonesian chicken Semen so the study aims to determine the level of skim milk in the best coconut water that can maintain the quality Spermatozoa Chicken Kampong during storage at a temperature of 5°C.

B. Methodology

1. The Material

The Semen used is derived from 5 Kampung chickens that are kept in an individual cage measuring 40 x 50 x 70 cm and fed with egg laying chickens as much as 150 gr/tail/day.

2. Research Procedures

a) Diluent making

The diluent is made by mixing skim milk (Tropicana Slim) with old coconut water. The percentage of skim milk used is 0%, 6%, 9% and 12% (table 1).

Table 1. Composition of Semen Diluent

Component	Treatments				
	P1	P2	P3	P4	P5
Coconut water (ml)	-	100	94	91	88
Skim Milk (GR)	-	-	6	9	12
Penicillin (IU/ml)	-	1000	1000	1000	1000
Streptomycin (mg/ml)	-	1	1	1	1

Diluent that has been made next added tris hydroxyl aminomethane to adjust the pH of diluent with chicken semen pH.

b) Semen collection

Collection or semen shelter is done using the method of sorting (masase). Sorting is done on the section of the cloaca until the papilla stands out and releases Semen. Furthermore, the Semen is accommodated using a spoit 1 ml.

c) Semen evaluation

Freshly collected Semen is evaluated macroscopic and microscopic in the laboratory. The macroscopic evaluation includes volume, pH, consistency, and color of Semen.

The microscopic evaluation involves the mass movements observed using a 10 x 10 magnification light microscope, with the assessment being excellent (+ + +), both (+ +), well (+), and bad (0). The motility of spermatozoa was observed using a light microscope with a magnification of 10 x 40. The percentage of motility was judged subjectively by comparing the living spermatozoa moving forward (progressive) with an unprogressive one. The rating is given from 0% (off all) to 100% (Motil all). The percentage of living spermatozoa (viability) is done using the dye of eosin-Negrosin, the review preparation is heated in the heating table for 10-15 sec, then examined under the light microscope with a magnification of 10 x 40 against ten

views. The concentration of spermatozoa is calculated using the Neubauer chamber with a 3% NaCl diluent. Percentage of spermatozoa which abnormalities; Observed using a light microscope with a magnification of 10 x 40.

d) Dilution, storage and evaluation of liquid Semen

Semen is divided into five tubes (without Diluent, Diluent with skim milk level 0%, 6%, 8%, and 12%), the ratio of Semen and Diluent used is 1:5, liquid Semen is then stored at a temperature of 5 oC. The observation of motility and viability was carried out at the 0, 12, 24, 36, and the 48 hours.

3. Data Analysis

The study used a complete randomized draft (CRD) with five treatments (Diluent) and three times repeated (Semen collection). If the treatment affects significantly, then proceed with the Duncan test.

C. Result and Discussion

1. Characteristics of fresh Semen chicken Village

Table 2. Characteristics of fresh Semen chicken Village

Parameters	Average \pm SE
Volume of Semen (ML)	0,09 \pm 0,02
Semen color	Milky white
Semen consistency	Thick
Semen pH	7 \pm 0,00
Concentration of spermatozoa (bn/ml)	2,65 \pm 0,28
Concentration of spermatozoa per Ejakulat (BN)	0,24 \pm 0,06
Spermatozoa mass Movement	+++
Motility of spermatozoa (%)	85,67 \pm 5,81
Spermatozoa viability (%)	99,73 \pm 0,13
Spermatozoa abnormalities (%)	13,84 \pm 0,20

The results showed that the volume rate of Semen produced was 0.09 \pm 0.02 ml/ejaculate, and the pH of Semen 7 (neutral), thick and white in milk. Previous research found that the volume of Semen chickens 0.19 ml (Murcahyana, Susilawati, & Isnaini, 2016) and 0.27 ml (Rahayu, Aji, Nurkhaffah, Fauziyah, & Annisa, 2017). Age and climate factors can cause a low amount of Semen in this research. According to (Zhang, Berry, McDaniel, Roland, Liu, Calvert, & Wil-hite, 1999), the amount of Semen and the concentration of chicken spermatozoa decreased with increasing age. The Semen pH produced in this study is classified as neutral (7) as reported by Rahayu et al., (2017); Wiyanti, Isnaini, & Trisunuwati (2013) and Danang, Isnaini, & Trisunuwati (2012).

The concentration of spermatozoa chickens produced in this study was 2.65 billion/ml of Semen, while 240 million spermatozoa were presented in a single ejaculation. This result is almost the same as the Semen concentration of Kampung chickens obtained by Murcahyana et al. (2012) namely 2.46 billion/ml and Tethool, Ollong, & Koibur (2017), 0.78-2.79 billion/ml, even higher than the obtained Lubis (2011) IE 1.6 billion/ml. Movement The resulting masses of spermatozoa are excellent (+ + +) indicating that spermatozoa had a massive wave of worship and rapidly shifting places.

The motility of spermatozoa acquired in this study included a typical category of 85.67 \pm 5.81%. The motility of spermatozoa in this study is higher than that reported by Tethool et al. (2017), i.e., 68.13%, also higher than Lubis (2011), Danang (2012) and Wiyanti et al. (2013) which only get 77% motility. But it is almost the same as the one obtained by Indrawati, Bebas, & Trilaksana, (2013) and Murcahyana et al. (2016) which earned 89% and 83.7% respectively. Viability (percentage of life) of spermatozoa of Chicken Kampong obtained at the research is very high (99.73 \pm 0.13%) Compared with previous analysis of 79.94% (Tethool et al., 2017), 83.87% (Lubis, 2011), 85.3% (Murcahyana et al., 2016) and 92% (Danang, 2012; Wiyanti et al., 2013; Indrawati et al., 2013).

The percentage of spermatozoa abnormalities in the research is quite high that is 13.84 \pm 0.20% when compared with the results reported by Murcahyana et al. (2016), Lubis (2011) and Wiyanti et al. (2013), which each get 8.7%, 6.8%, and 5.1%. The type of abnormality seen in this study is generally a secondary abnormality, namely abnormalities in the tail. According to

Feradis (2010), secondary defects occur outside Tubuli seminiferous during ejaculation due to overheating or excessive cooling or contaminated water, urine, and antiseptic.

2. *Motility and viability of Spermatozoa chicken village with various levels of milk Skim in Diluent*

Table 3. Motility Spermatozoa Chicken Village During Storage with Various Levels of Skim Milk in Semen Diluent

ST (Hour) ^a	Skim Milk Level				
	WD ^b	0%	6%	9%	12%
(%).....				
0	86,96±2,12 ^{bc}	60,60±4,70 ^a	93,16±2,38 ^c	93,36±1,81 ^c	78,57±1,87 ^b
12	29,83±1,48 ^a	22,33±3,71 ^a	83,53±2,77 ^c	76,9±1,57 ^c	48,85±1,79 ^b
24	0,00±0,00 ^a	10,33±2,02 ^b	79,41±3,60 ^e	69,87±2,07 ^d	36,59±1,63 ^c
36	0,00±0,00 ^a	1,66±1,66 ^a	64,06±3,46 ^d	52,61±1,20 ^c	25,95±2,61 ^b
48	0,00±0,00 ^a	0,00±0,00 ^a	49,70±6,02 ^c	43,73±4,48 ^c	15,57±3,90 ^b

Description: The numbers followed by different letters on the same line state the genuine difference ($P < 0.01$). ST^a: Storage Long, WT^b: without Diluent.

Table 4. Viability of Spermatozoa Chicken Village during Storage with Various Levels of Skim Milk in Semen Diluent

ST (Hour) ^a	Skim Milk Level				
	WD ^b	0%	WD ^b	9%	WD ^b
(%).....				
0	95,79±4,02 ^a	87,83±10,58 ^a	99,07±0,86 ^a	98,71±1,26 ^a	90,36±4,56 ^a
12	36,46±1,81 ^a	33,25±8,72 ^a	88,09±2,07 ^c	87,53±3,95 ^c	56,67±3,08 ^b
24	0,00±0,00 ^a	21,34±4,46 ^b	83,28±4,86 ^d	79,90±3,44 ^d	45,68±2,24 ^c
36	0,00±0,00 ^a	2,83±2,48 ^a	64,06±3,46 ^d	52,61±1,20 ^c	25,95±2,61 ^b
48	0,00±0,00 ^a	0,00±0,00 ^a	49,70±6,02 ^c	43,73±4,48 ^c	15,57±3,90 ^b

Description: The numbers followed by different letters on the same line state the genuine difference ($P < 0.01$). ST^a: Storage Long, WT^b: without Diluent.

The results showed that the use of various diluent Diluent was very noticeable ($P < 0.01$) against the motility and viability of the Kampong Chicken spermatozoa at every 12 hours of storage (table 3 and table 4). At the 24th hour shows that without diluent all spermatozoa have experienced death whereas with the use of Diluent containing 100% of the spermatozoa coconut water can still last a little longer although not as good as milk Diluent Skim. It was due to the presence of several nutrients in coconut water that support the life of spermatozoa during storage.

According to Reddy & Lakshmi (2014), coconut water contains 95% of the water, 5% sugar consisting of glucose, fructose, and sugars. The content of glucose, fructose, and sucrose can be utilized as an energy source for spermatozoa during storage. Coconut water is rich in minerals (electrolytes) such as potassium, calcium, magnesium, manganese, and small amounts of sodium (Reddy & Lakshmi, 2014). Potassium and sodium function to maintain the electrolyte balance in Semen.

Spermatozoa cells are very quickly exposed to lipid peroxidation by free radicals consisting of hydrogen peroxide, superoxide anions, and hydroxyl radicals that can cause damage to the membrane of spermatozoa during anaerobic storage (Sinha, Sinha, & Singh, 1996). The occurrence of cold shock in spermatozoa during cold storage is associated with oxidative stress caused by free radicals (Sanocka & Kurpisz, 2004; and Thuwanuta, Chatdarongb, & Berqvista, 2011). Azawi & Hussein (2013) stated that free radicals could largely be eliminated by the presence of antioxidants.

Coconut water contains antioxidants such as phenol and ascorbic acid (vitamin C) (Santos, Vanderson, Bispo, Adriano, Filho, Isabella, Pinto, Lucas, Danta, Daiane, Vasconcelos, Fabúla, Abreu, Danilo, Isaac, Florêncio, Osmar, Marisa, Medeiros, & Humberto, 2013) so that it can protect the cells of spermatozoa from free radicals. According to Santos et al. (2013), coconut water is better able to reduce the concentration of ROS when compared to vitamin C and coconut water also effectively protects the fibroblast from the adverse effects of hydrogen peroxide.

The high motility of spermatozoa is found in the treatment of coconut water thinning with a skim milk level of 6%, and a skim milk level of 9% is 93,16-93,36% in the hour storage of the 0, 76,9-83,53% in the 12th hour, and 30,84-36,87% in the hour of 48. While the highest motility in the 24th hour and the 36 hours are in the treatment with skim milk level 6% (79.41%), the viability of spermatozoa in hour 0 does not differ between treatments, but during the general storage of spermatozoa, viability can withstand better on Diluent with skim milk level 6% and 9%.

The high motility and viability of spermatozoa on the treatment with the addition of skim milk were suspected because of the protein content of casein and lactose in milk that keeps the survival of spermatozoa during storage. Naturally, there are binder proteins (binders) that have particular functions. According to Manjunath (2012) that BSP protein (Binder of Sperm) could harm spermatozoa during storage. It was due to the BSP protein able to manage the spermatozoa by releasing lipid compounds in the cell membrane of spermatozoa while the presence of casein on milk Diluent can interact with BSP to decrease the BSP strap on the cell membrane, thus preventing the loss of lipid compounds from the cell membrane of spermatozoa.

The presence of lactose in skim milk is allegedly a source of energy for the movement of spermatozoa during storage. Movement activity (motility) of spermatozoa requires energy derived from changes in ATP (Adenosine Triphosphate) to ADP (Adenosine in Phosphate) and AMP (Adenosine monophosphate) (Garner & Hafez, 2000). Also, lactose plays a role in protecting spermatozoa. It was by the opinion of Watson (1990), stating that lactose has low cellular permeability and is believed to preserve spermatozoa by acting extracellular.

Skim milk has been regarded as a non-enzymatic antioxidant due to the presence of sulphhydryl clusters (Bustamante-Filho, Pederzolli, Sgaravatti, Gregory, Dutra-Filho, Jobim, & Mattos, 2009). During storage, spermatozoa consume oxygen and oxygen metabolism; the result of the metabolism is ROS (Radical Oxygen Scavenging) (Agarwal, Saleh, & Bedaiwy, 2003). The accumulation of ROS can occur during storage of Semen when there is no antioxidant in Semen Diluent. ROS caused the occurrence of oxidative stress (Michael, Alexopoulos, Pontiki, Hadjipavlou-Litina, Saratsis, Ververidis, & Boscov, 2008). Generally, antioxidants prevent damage by intermediates of oxidants by free radicals or the reactive metabolites of the antioxidant itself. It can reduce the impact of oxidative stress on spermatozoa during storage and improve the quality of spermatozoa in liquid Semen (Storey, 1997).

The use of skim milk level 12% into diluent significantly shows the motility and lower spermatozoa viability when compared to 6% and 9%, this may be caused by skim milk concentrations too high that cause Disturbances in spermatozoa. It was by the opinion of Songsasen, Murton, Paccamonti, Eilts, Godke, & Leibo (2002) stating that the level of skim milk that is too high can cause hypertonicity in Diluent by removing water from the cells and lowering motility and viability.

Skim milk 6% and 8% in this research were suspected to be better able to meet the needs of spermatozoa nutrients and antioxidant needs during storage for up to 48 hours. So if applied to the field, the Diluent deserve to be used as a Semen Diluent stored at a temperature of 5°C for 36-48 hours before being inseminated to the female chickens.

D. Conclusion

The Diluent of coconut water with skim milk level 6% and 9% can maintain the quality of the Kampong Chicken spermatozoa better during storage of 48 hours at a temperature of 5°C.

E. References

- Agarwal, A., Saleh, R. A., & Bedaiwy, M. A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil. Steril. Vol. 79*, pp. 829-843.
- Azawi, O.I., & Hussein, E.K. (2013). Effect of vitamin C or E supplementation to tris diluent on the semen quality of Awassi rams preserved at 5 °C. *Veterinary Research Forum. Vol. 4* (3), pp. 157-160.
- Azizah, & Arifiantini, R. I. (2009). Kualitas semen beku kuda pada pengencer susu skim dengan konsentrasi gliserol yang berbeda. *Jurnal Veteriner. Vol. 10* (2), pp. 63-70.
- Baba, R. A., & Qistina, M. N. N. (2015). Preliminary evaluation on the use of coconut water as semen diluent for local Kampung cockerel. *Proceed 2nd Asian Regional Conference on Animal production (ARCAP) dan 36th the Animal Conference of Malaysia Society of Animal (MSAP)*. 1-3 Juni, 2015. Part Dickson Negeri Sembilan, Malaysia.

- Bustamante-Filho, I. C., Pederzoli, C. D., Sgaravatti, A. M., Gregory, R. M., Dutra-Filho, C. S., Jobim, M. I. M., & Mattos, R. C. (2009). Skim milk-egg yolk based semen extender compensates for nonenzymatic antioxidant activity loss during equine semen cryopreservation. *Anim. Reprod. Vol. 6*, pp. 392-399.
- Danang, D. R., Isnaini, N., & Trisunuwati, P. (2012). Pengaruh lama simpan semen terhadap kualitas spermatozoa ayam kampung dalam pengencer ringer's pada suhu 4°C. *J Ternak Tropika. Vol. 13* (1), pp. 47-57.
- Dwitarizki, N. D., Ismaya, & Asmarawati, W. (2015). Pengaruh pengenceran sperma dengan air kelapa dan aras kuning telur itik serta lama penyimpanan terhadap motilitas dan viabilitas spermatozoa domba garut pada penyimpanan 5°C. *Buletin Peternakan. Vol. 39* (3), pp. 149-156.
- Feradis. (2010). *Bioteknologi Reproduksi Pada Ternak*. Bandung, Indonesia: Alfa Beta.
- Garner, D. L., & Hafez, E. S. E. (2000). Spermatozoa and seminal plasma. In: Hafez, E.S.E., dan Hafez, B., (Editor). *Reproduction in Farm Animals*. 7th edition. Philadelphia, USA: Lippincott Williams and Wilkins.
- Hernawati, T., Fevianita, D. H., Hariadi, M., & Kurnijasanti, R. (2010). Viabilitas dan motilitas spermatozoa entok (*Cairina moschata*) dalam kombinasi bahan pengencer susu skim, fruktosa dan kuning telur. *Veterinaria Medika Vol. 3* (1), pp. 49-52.
- Hoppe, C., Andersen, G. S., Jarobsen, S., Molgaard, C., Friis, H., Sangild, P. T., & Michaelsen, K. F. (2008). The use of whey or skimmed milk powder in fortified blended foods for vulnerable groups. *Journal of nutrition. Vol. 138*, pp. 145-161.
- Indrawati, D., Bebas, W., & Trilaksana, I. G. N. B. (2013). Motilitas dan Daya Hidup Spermatozoa Ayam Kampung dengan Penambahan Astaxanthin pada Suhu 3- 5o C. *Indonesia Medicus Veterinus. Vol. 2* (4), pp. 445 - 452.
- Kewilaa, A. I., Ondho, Y. S., & Setiatin, E. T. (2013). Pengaruh berbagai jenis pengencer air kelapa muda dengan penambahan kuning telur yang berbeda terhadap kualitas spermatozoa semen cair domba ekor tipis (DET). *Agrianimal. Vol. 3*(1), pp. 1-9.
- Lubis, T. M. (2011). Motilitas spermatozoa ayam kampung dalam pengencer air kelapa, NaCl fisiologis dan air kelapa-NaCl fisiologis pada 25-29 °C. *Agripet. Vol. 11* (2), pp. 45-50.
- Manjunath, P. (2012). New insight into the understanding of the mechanism of sperm protection by extender components. *Anim. Reprod. Vol. 9* (4), pp. 809-815.
- Michael, A. J., Alexopoulos, C., Pontiki, E. A., Hadjipavlou-Litina, D. J., Saratsis, P., Ververidis, H. N., & Boscos, C. M. (2008). Quality and reactive oxygen species of extended canine semen after vitamin C supplementation. *Theriogenology. Vol. 70*, pp. 827-835.
- Murcahyana, Susilawati, T., & Isnaini, N. (2016). Pengaruh pemberian ekstrak buah mengkudu (*Morinda citrifolia*) dalam larutan natrium klorida fisiologis sebagai bahan pengencer semen terhadap peningkatan kualitas spermatozoa ayam buras pada suhu ruang. *Jurnal Kedokteran Hewan. Vol.10* (2), pp. 175-180.
- Rahayu, A., Aji, L. P. W., Nurkhaffah, P. S., Fauziah, A., & Annisa, D. N. (2017). Evaluasi kualitas spermatozoa ayam kampung (*Gallus domesticus*) setelah penambahan pakan ayam rempah-rempah alami. *Seminar Nasional Peternakan 3*; 18 September 2017; Makassar, Universitas Hasanuddin. Hlm 43-52.
- Reddy, E.P., & Lakshmi, T.M., (2014). Coconut water-properties uses nutritional benefits in health and wealth and health and disease: a review. *Journal of Current Trends in Clinical Medicine and Laboratory Biochemistry. Vol. 2* (2), pp. 6-18.
- Sanocka, D. M., & Kurpisz, M. (2004). Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol. Vol. 2*, pp.12-18.
- Santos, J. L. A., Vanderson, S., Bispo, Adriano, B. C., Filho, Isabella, F. D., Pinto, Lucas, S., Danta, Daiane, F., Vasconcelos, Fabúla, F., Abreu, Danilo, A. M., Isaac, A. M., Florêncio, P. F., Osmar, F. G., Marisa H. G., Medeiros, & Humberto R. M. (2013). Evaluation of Chemical constituents and antioxidant activity of coconut water (*Cocos nucifera* L.) and caffeic acid in cell culture. *Annals of the Brazilian Academy of Sciences. Vol. 85* (4), pp. 1235-1246.
- Sinha, M. P., Sinha, A. K., & Singh, B. K. (1996). The effect of glutathione on the motility, enzyme leakage, and fertility of frozen goat semen. *Theriogenology. Vol. 41*, pp. 237-243.
- Songsasen, N., Yu, I., Murton, S., Paccamonti, D. L., Eilts, B. E., Godke, R. A., & Leibo, S. P. (2002). Osmotic sensitivity of canine spermatozoa. *Cryobiology. Vol. 44*, pp. 79-90.
- Storey, B. T. (1997). Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Mol. Hum. Reprod. Vol. 3*, pp. 203-213.

- Tethool, A. N., Ollong, A. R., & Koibur, J. F. (2017). Kualitas mikroskopik spermatozoa ayam kampung (*Gallus gallus*) setelah pemberian sari buah merah (*Pandanus conideus* LAM). *Seminar Nasional Peternakan 3*; 18 September 2017; Makassar, Universitas Hasanuddin. Hlm 77-84.
- Thuwanuta, P, Chatdarongb, K, & Berqvista, A. S. (2011). The effects of antioxidants on semen traits and in vitro fertilizing ability of sperm from the flat-headed cat (*Prinailurus planiceps*). *Theriogenology*. Vol. 76, pp.115-125.
- Watson, P. F. (1990). Artificial insemination and the preservation of semen. In: Lamming GE (Editor). *Marshall's Physiology of Reproduction*. Edinburgh, USA: Churchill-Livingstone.
- Wiyanti, D. B., Isnaini, N., & Trisunuwati, P. (2013). Pengaruh lama simpan semen dalam pengencer NaCl fisiologis pada suhu kamar terhadap kualitas spermatozoa ayam kampung (*Gallus gallus domesticus*). *Jurnal Kedokteran Hewan*. Vol. 7 (1), pp. 53-55.
- Yatusholikhah, I., Isnaini, N., & Ihsan, M. N. (2015). Pengaruh pengencer skim milk dengan berbagai level fitrat kecambah kacang Hijau (*Phaseolus radiates* L.) terhadap kualitas semen cair Sapi Simmental pada suhu ruang. *J. Ternak Tropika* Vol. 16, (2), pp. 16-24
- Zhang, X., Berry, W. D., McDaniel, G. R., Roland, D. A., Liu, P., Calvert, C., & Wil-hite, R. (1999). Body weight and semen production of broiler breeder males as influenced by crude protein levels and feeding regimens during rearing. *Poult. Sci.* Vol. 78, pp. 190-196.