

The Effect of Egg Yolk Diluter On Semen Quality in Different Chicken Breeds at Poultry Breeding Center Temanggung-Central Java

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Abstract:-The objective of this study was to determine the effect of different level of NaCl and egg yolk on sperm motility in different breeds of chicken (Lingnan, Bangkok, Kedu, and Arabic chicken). The semen of different breeds were diluted with NaCl and egg yolk. Treatment comprised of A treatment as a control (NaCl); treatment B (NaCl + 10% egg yolk), treatment C (NaCl + 15% egg yolk), and treatment D (NaCl + 20% egg yolk). The sperm motility was encountered after 0 ; 30 ; 60 and 120minute of storage in room temperature. The research was arranged in a completely randomized design with fourtreatments, and five replications.The results of sperm motility each breed showed different result. The sperm motility showed no significantly in Lingnan chicken ($F = 2.033$, $p = 0.116$); Arabic chicken ($F = 2.704$, $p = 0.051$). While siginificantly was observed in Bangkok chicken ($F = 2.845$, $p = 0.043$) and Kedu chicken ($F = 6.134$, $p = 0.001$). The different breeds shown that the treatment interaction with breed was no significant on sperm motility ($F = 0.662$, $p = 0.743$) and Lingnan chicken has had the highest sperm motility. According to this experiment, it can be concluded that semen dilution with egg yolk could not maintain the quality of semen and a certain high percentage of egg yolk and longtime storage will decreased sperm motility.

Key words: Egg yolk, semen motility, chicken breeds.

I. BACKGROUND

Lingnan chicken is a local chicken breed from China, with adult body weight reach between 3,5–4 kg, with the height of rooster approximately 80 cm and the hen is 60 cm. The feather colour is golden yellow with the combination of red, and their shankis yellow. The Lingnan chicken has better body endurance compared to the local chicken from red Arabian chicken in the aspect of its adaptation in the surrounding temperature and body resistance to the diseases and illness. Lingnan chicken has softer meat texture compared to the Indonesian local chicken (Kedu and Bangkok breed/inheritance), however it is more solid than the broiler chicken. The egg productivity of Lingnan chicken can reach 50% hen/day or 150–180 eggs/year.As the alternative of local chicken substitution, Lingnan chicken is hopefully be able to supply the lack of local product to fulfill the demand of local chicken meats and eggs.A good quality of semen diluter is needed as one of requirement to achieved successfull in an artificial insemination of chicken.One of kind diluter is egg yolk. The benefit of egg yolk is contain lipoprotein and lecithin and has function to preserve and protect the integrity surrounding spermatozoa lipoprotein. Besides that, the egg yolk is easy and cheap to get (Negoro, 2011). The effect of the egg yolk as diluterinLingnan, Arabian, and Local chicken semen needs to be further studies.

Local chicken is original Indonesian breed by which crossed with jungle fowl (*Gallus bankiva*) and widespread throughout the islands of Java and NusaTenggara (*Gallus varius*) and are not directed to a specific production purpose (Budipurwanto, 2001). According to Blakly and Bade(1994) that the ancestors of the local chicken is red jungle fowl (*Gallus gallusor Red jungle fowl*).

II. MATERIALS AND METHODS

This research performed in Poultry Breeding Center-Temanggung and Laboratory of Animal Genetics-Breeding and Reproduction the Faculty of Animal Science and Agriculture, Diponegoro University.

III. RESEARCH MATERIALS

The material that used during this research is semen collected from 4 different males of Lingnan, Arabian, Kedu and Bangkok crossbred. The material used are NaCl as basic diluter, egg yolk, aquabidest, eosin 0.2%, test tube and antibiotic. The tools that used are test tube to process the semen collecting, water heater, scaled tube to figure out the volume of the semen that can be produce, universal indicator paper to find out the acidic degree, microscope to observe the motility, object glass, deck glass, and bunsen, beaker glass, filter paper, glass measurement to dilute the semen, and stationery to note the acquiring results.

Research Method

Method that used in the data collection consists of several steps, those are:

- Research preparation phase includes the preparation of the material and preparation tools.
- Material procurement. Material procurement begun with preparing for the male chicken in order to collecting sperm. After that, held the semen storage preparations.
- Adaptation process. The process of adaptation include adaptive the male cock for semen collection using massage method in order to get out semen, semen storage carried out in the morning.
- Equipment preparation. Preparation equipment is cleaning up all the equipments, and preparing research materials.

Data collection process, the parameters that will be used are the volume of semen, pH, semen colour, motility. Spermatozoa motility can be measured by dropping the semen in the middle of object glass than cover it with deck glass, and observe it under the microscope with magnificent of 10 x 40.

IV. RESEARCH DESIGN AND DATA ANALYSIS

The research was arranged in a completely randomized design five replications. The treatments on four breeds with dilution NaCl and Egg Yolk. Treatment A was control with dilution NaCl. Treatment B (NaCl + 10% Egg Yolk), C (NaCl + 15% Egg Yolk), and D (NaCl + 20% Egg Yolk). The hypothesis was tested with analysis of univariate variance, while mean comparison after significant treatment effects was done with Duncan Multiple Range Test. Data were analyzed by F test range. If there is a significant treatment effect, will then followed by the Duncan multiple test area to determine the differences between treatments. Criteria for decision-making on the level of 95% or $\alpha = 0.05$ if $F_{count} > F_{Table}$ then H_0 is rejected and H_1 accepted.

V. RESULTS AND DISCUSSION

Semen Volume of Lingnan, Bangkok, Kedu and Arabic Chicken

Result on chicken semen volume known that average of semen volume of Lingnan chicken is 0.76 ml/ejaculation, Bangkok chicken is 0.48 ml/ejaculation, Kedu chicken is 0.48 ml/ejaculation, and Arabic chicken is 0.18 ml/ejaculation. From all breeds, Arabic has lowest semen volume and Lingnan has highest of semen volume (see Table 1).

Semen volume for Indonesian chicken still in the normal range for a chicken ejaculation. According to Rose (1997) roosters produce 0.5-1.0 ml of semen per ejaculation. Sperm volume for Arabic chicken also is relatively normal for a chicken ejaculation Arabic chicken. According Nataamijaya, *et. al.* (2003) that the volume of ejaculate is 0.26 ml with creamy white cement. Isnaini (2000) also found semen obtained Arabic chicken in a fresh state has semen volume 0.24 ml and pH value 7.4.

Based on analyzed paired T-test there is no differences ($p > 0.05$) between semen volume of Lingnan chicken and Bangkok chicken. Paired t-test between Lingnan chicken and Kedu chicken shown there is no differences ($p > 0.05$) between semen volume Lingnan chicken and Kedu chicken. Paired t-test between Lingnan chicken and Arabic chicken shown there is differences ($p < 0.05$) between semen volume Lingnan chicken and Arabic chicken. Paired t-test between Bangkok chicken and Kedu chicken shown there is no differences ($p > 0.05$) between semen volume Bangkok chicken and Kedu chicken. Paired t-test between Bangkok chicken and Arabic chicken shown there is differences ($p < 0.05$) between semen volume Bangkok chicken and Arabic chicken. Paired t-test between Kedu chicken and Arabic chicken shown there is differences ($p < 0.05$) between semen volume Kedu chicken and Arabic chicken. Based on analyzed paired t-test no differences between Lingnan, Bangkok, and Kedu chicken it means that there is no differences on semen production of that breed, but difference on Arabic chicken has lowest semen production compare all breeds.

Table 1. Semen Volume in Lingnan, Bangkok, Kedu and Arabic Chicken

Replication	Breeds of Chicken			
	Lingnan	Bangkok	Kedu	Arabic
1	0.5	0.8	0.5	0.2
2	1.0	0.4	0.6	0.1
3	0.5	0.4	0.5	0.3
4	0.9	0.4	0.3	0.1
5	0.9	0.4	0.5	0.2
Total (Σ)	3.8	2.4	2.4	0.9
Average	0.76	0.48	0.48	0.18

Semen Concentration of Lingnan, Bangkok, Kedu and Arabic Chicken

According to semen concentration (Table 2), shown that average of semen concentration of Lingnan chicken is 259.8×10^7 /ml, Bangkok chicken is 185×10^7 /ml, Kedu chicken is 270.6×10^7 /ml, and Arabic chicken is 245.2×10^7 /ml. From all breeds, Bangkok chicken has lowest semen concentration and Kedu chicken has highest of semen concentration.

Table 2. Semen Concentration in Lingnan, Bangkok, Kedu and Arabic Chicken

Replication	Breeds of Chicken			
	Lingnan	Bangkok	Kedu	Arabic
1	246×10^7	190×10^7	205×10^7	247×10^7
2	222×10^7	126×10^7	308×10^7	205×10^7
3	185×10^7	153×10^7	171×10^7	239×10^7
4	363×10^7	236×10^7	324×10^7	334×10^7
5	283×10^7	220×10^7	345×10^7	201×10^7
Total (Σ)	1299×10^7	925×10^7	1353×10^7	1226×10^7
Average	259.8×10^7	185×10^7	270.6×10^7	245.2×10^7

Semen concentration varied from thin and thick suspension. Semen concentration for Arabic chicken also is relatively normal. Isnaini (2000) showed that the semen of Arabic chickens in the fresh condition has the concentration of 216×10^6 /ml.

Based on analyzed paired t-test there is no differences ($p > 0.05$) between semen concentration of Lingnan chicken and Bangkok chicken. Paired t-test between Lingnan chicken and Kedu chicken shown there is differences ($p < 0.05$) between semen concentration Lingnan chicken and Kedu chicken. Paired t-test between Lingnan chicken and Arabic chicken shown there is no differences ($p > 0.05$) between semen concentration Lingnan chicken and Arabic chicken. Paired t-test between Bangkok chicken and Kedu chicken shown there is no differences ($p > 0.05$) between semen concentration Bangkok chicken and Kedu chicken. Paired t-test between Bangkok chicken and Arabic chicken shown there is no differences ($p > 0.05$) between semen concentration Bangkok chicken and Arabic chicken. Paired t-test between Kedu chicken and Arabic chicken shown there is differences ($p < 0.05$) between semen concentration Kedu chicken and Arabic chicken.

Effect of Diluter on Sperm Motility of Lingnan Chicken

Lingnan chicken showed that the highest means of motility showed on control treatments and decreased on treatments with 10% egg yolk and 15% egg yolk and then decreased on treatments with 20% egg yolk. For more details can be seen in the **Illustration 1**.

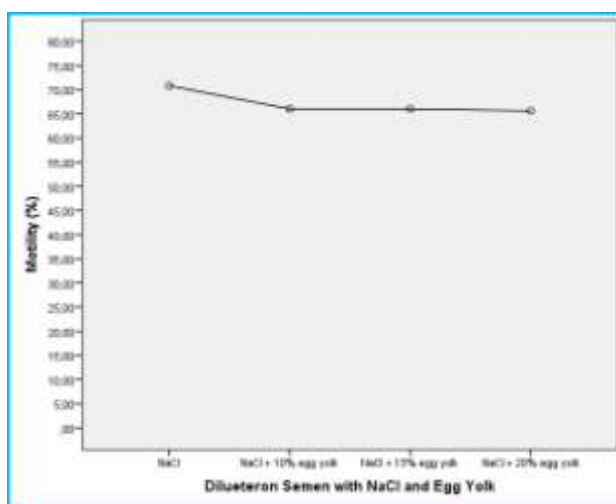


Illustration 1. Graph of Sperm Motility in Lingnan Chicken

F value for treatment dilution of NaCl + Egg Yolk is 2.300 <F Table 2.719 with Sig. 0.084 > 0.05 it means that the treatments dilution semen with NaCl + egg yolk did not significantly affect to sperm motility of Lingnan chicken. From the Table 3 shown that F value for time is 30.459 >F Table 2.486 with Sig. 0.000 < 0.05 it means that time significantly affect to sperm motility of Lingnan chicken. Still on the table below the F value for interaction treatments and time is 0.209 <F Table 1.875 with Sig. 0.998 > 0.05 it means that interaction of

treatments dilution and time did not significantly affect to sperm motility of Lingnan chicken. For more detail can be seen on the Table 3.

Table 3. The F-tests Between-Subjects Effects of Sperm Motility in Lingnan Chicken

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3598,051 ^a	19	189,371	6,908	,000
Intercept	305753,702	1	305753,702	11152,772	,000
Dilution	189,153	3	63,051	2,300	,084
Time	3340,123	4	835,031	30,459	,000
Dilution * Time	68,775	12	5,731	,209	,998
Error	2193,203	80	27,415		
Total	311544,956	100			
Corrected Total	5791,254	99			

a. R Squared = ,621 (Adjusted R Squared = ,531)

Effect of Diluter on Sperm Motility of Bangkok Chicken

From the data means of motility semen Bangkok chicken showed that the highest means of motility showed on control treatments and decreased on treatments with 10% egg yolk, increase on 15% egg yolk and then decreased on treatments with 20% egg yolk. For more details can be seen in the Illustration 2.

Based on F Test is known that F value for treatment dilution of NaCl + Egg Yolk is 3.116 >F Table 2.719 with Sig. 0.031 < 0.05 it means that the treatments dilution semen with NaCl + egg yolk significantly affect to sperm motility of Bangkok chicken. From the Table 4 shown that F value for time is 43.941 >F Table 2.486 with Sig. 0.000 < 0.05 it means that time significantly affect to sperm motility of Bangkok chicken. Still on the table below the F value for interaction treatments and time is 0.641 <F Table 1.875 with Sig. 0.801 > 0.05 it means that interaction of treatments dilution and time did not significantly affect to sperm motility of Bangkok chicken. For more detail can be seen on the **Table 4.**

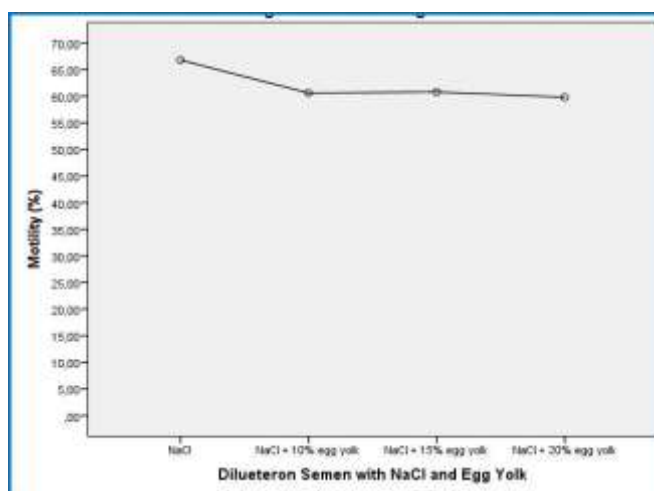


Illustration 2. Graph of Sperm Motility in Bangkok Chicken

Table 4. The F-tests Between-Subjects Effects of Sperm Motility in Bangkok Chicken

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6531,919 ^a	19	343,785	10,147	,000
Intercept	272568,571	1	272568,571	8045,200	,000
Dilution	316,715	3	105,572	3,116	,031
Time	5954,756	4	1488,689	43,941	,000
Dilution * Time	260,448	12	21,704	,641	,801
Error	2710,372	80	33,880		
Total	281810,862	100			
Corrected Total	9242,291	99			

After F test followed by linear regression. Based on the linear regression equation is $Y = 64.895 + (-1.335) X_1 + (-0.178) X_2$. It means that if the treatments NaCl + egg yolk for 0 % of the value of sperm motility is 64.895. And if the treatment NaCl + egg yolk increased by 1 unit (5%) the amount of sperm motility will decrease by 1.335. And if the time increased by 1 unit (30 minute) the amount of sperm motility will decrease by 0.178. Based on the output coefficient of determination is known that the R^2 value is 0.642 it means that the variable treatment dilution semen with NaCl + Egg yolk give the effect of 64.2% and others affected 35.8% by other factors not examined in this study.

Effect of Diluter on Sperm Motility of Kedu Chicken

From the data means of motility semen Kedu chicken showed that the highest means of motility showed on control treatments and decreased until treatments 20% egg yolk. For more details can be seen in the **Illustration 3**.

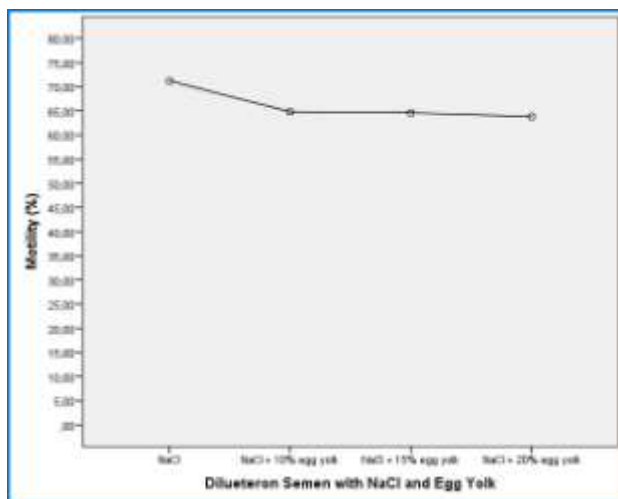


Illustration 3. Graph of Motility Sperm Kedu Chicken

Based on F Test is known that F value for treatment dilution of NaCl + Egg Yolk is 6.175 > F Table 2.719 with Sig. 0.001 < 0.05 it means that the treatments dilution semen with NaCl + egg yolk significantly affect to sperm motility of Kedu chicken. From the Table 5 shown that F value for time is 61.187 > F Table 2.486 with Sig. 0.000 < 0.05 it means that time significantly affect to sperm motility of Kedu chicken. Still on the table below the F value for interaction treatments and time is 1.447 < F Table 1.875 with Sig. 0.163 > 0.05 it means that interaction of treatments dilution and time did not significantly affect to sperm motility of Kedu chicken. For more detail can be seen on the Table 5.

Table 5. The F-tests Between-Subjects Effects on Sperm Motility in Kedu Chicken

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4925,199 ^a	19	259,221	14,770	,000
Intercept	299240,727	1	299240,727	17050,600	,000
Dilution	325,108	3	108,369	6,175	,001
Time	4295,399	4	1073,850	61,187	,000
Dilution * Time	304,693	12	25,391	1,447	,163
Error	1404,013	80	17,550		
Total	305569,939	100			
Corrected Total	6329,212	99			

Effect of Diluter on Sperm Motility of Arabic Chicken

From the data means semen motility of Arabic chicken showed that the highest means of motility showed on control treatments and decreased until treatments 20% egg yolk. For more details can be seen in the **Illustration 4**.

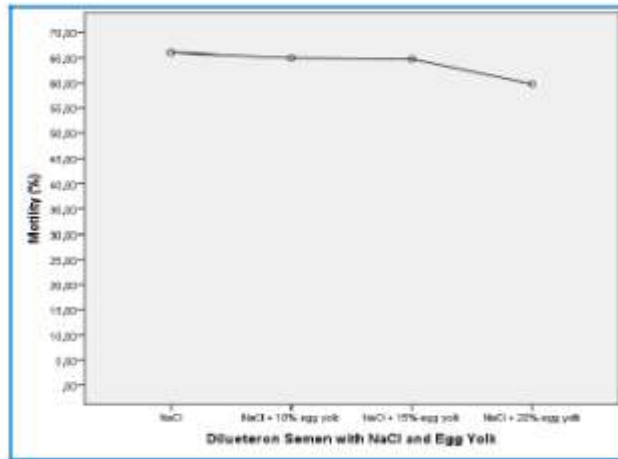


Illustration 4. Graph of Motility Sperm Arabic Chicken

Based on F Test is known that F value for treatment dilution NaCl + Egg Yolk is 2.763 >F Table 2.719 with Sig. 0.047 < 0.05 it means that the treatments dilution semen with NaCl + egg yolk significantly affect to sperm motility of Arabic chicken. From the Table 6 shown that F value for time is 13.000 >F Table 2.486 with Sig. 0.000 < 0.05 it means that time significantly affect to sperm motility of Arabic chicken. Still on the table below the F value for interaction treatments and time is 0.385 <F Table 1.875 with Sig. 0.965 > 0.05 it means that interaction of treatments dilution and time did not significantly affect to sperm motility of Arabic chicken. For more detail can be seen on the Table 6.

Table 6. The F-tests Between-Subjects Effects on Sperm Motility in Arabic Chicken

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1660,040	19	87,371	3,416	,000
Intercept	283194,266	1	283194,266	11072,893	,000
Dilution	211,979	3	70,660	2,763	,047
Time	1329,905	4	332,476	13,000	,000
Dilution * Time	118,156	12	9,846	,385	,965
Error	2046,036	80	25,575		
Total	286900,341	100			
Corrected Total	3706,076	99			

Effect of Diluter on Sperm Motility Compare to Different Breed

From the data means sperm motility of different breed showed that the highest means of sperm motility foundin Lingnan chicken, the second means of sperm motility showed in Kedu chicken, the third means of sperm motility showed on Arabic chicken and the last sperm motility is Bangkok chicken.

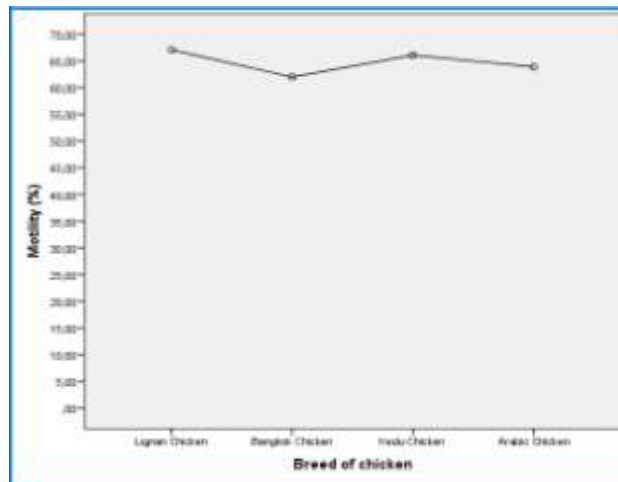


Illustration 5. Graph of Sperm Motility in Different Breed of Chicken

For more details can be seen in the Illustration 5. Based on F Test, it is known that F value for breeding is $7.550 > 2.633$ with Sig. $0.000 < 0.05$ it means that there significantly different on motility sperm in this research. F value for treatment dilution NaCl + Egg Yolk is $11.360 > F$ table 2.633 with Sig. $0.000 < 0.05$. It means that the treatments dilution semen with NaCl + egg yolk significantly affect to sperm motility on different breed. From the Table 7 shown that F value for time is $133.704 > F$ table 2.400 with Sig. $0.000 < 0.05$. It means that time significantly affect to sperm motility in different breed. Still on the Table 7, the F value for interaction different breeding and treatments dilution is $0.652 < 1.909$ with Sig. $0.752 > 0.05$, it means that interaction breeding and treatments did not significantly affect on motility sperm in different breed.

Table 7. The F-tests Between-Subjects Effects on Sperm Motility in Different Breed

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17306,522 ^a	79	219,070	8,392	,000
Intercept	1160165,952	1	1160165,952	44442,160	,000
Breed	591,313	3	197,104	7,550	,000
Diluet	889,682	3	296,561	11,360	,000
Time	13961,367	4	3490,342	133,704	,000
Breed * Diluet	153,273	9	17,030	,652	,752
Breed * Time	958,815	12	79,901	3,061	,000
Diluet * Time	245,500	12	20,458	,784	,667
Breed * Diluet * Time	506,571	36	14,071	,539	,987
Error	8353,624	320	26,105		
Total	1185826,098	400			
Corrected Total	25660,146	399			

F value for interaction different breed and time is $3.061 > 1.783$ with Sig. $0.000 < 0.05$. It means that interaction breed and time significantly affect on motility sperm in different breed. F value for interaction treatment dilution and time is $0.784 > 1.783$ with Sig. $0.667 > 0.05$ it means that interaction treatments dilution and did not significantly affect on sperm motility in different breed.

According to F value for interaction different breed, dilution treatments and time is $0.539 < 1.454$ with Sig. $0.987 > 0.05$ it means that interaction breed, dilution treatments and time did not significantly affect on sperm motility in different breed. For more detail can be seen on the Table 7.

Based on the results of study revealed that sperm motility in Lingnan, Bangkok chicken, Kedu chicken and Arabic chicken with dilution treatment using egg yolks, the greater of percentage egg yolk, sperm motility was lower along with the longer storage time. Egg yolks have a more complete nutritional composition than egg whites/albumin. The main composition of egg yolk is composed of water, protein, fat, carbohydrates, minerals, and vitamins (Sarwono, 1995) and include the perfect egg protein because it contains all the essential amino acids in quantities large enough (Haryanto, 1996). Moreover, according to Toelihere (1979), egg yolk containing lipoprotein and lichenin that maintain and protect the integrity of the cell envelope lipoprotein spermatozoa and prevents cold shock. According to Hafez (1974) in the discovery of Philip (1939), the use of chicken egg yolk as a semen diluent is precious and at this time has been used widespread throughout the world. But, in the egg yolks also contained substances that can damage the fertility of spermatozoa and can be toxic to spermatozoa, also substances that can prevent damage to spermatozoa during the cooling process (Situmorang, 1991). In current study however, sperm motility increased rapidly in all the egg yolks treatment at 120 minutes after storage. Comparison between sperm motility in different breed based on the results of the study, Bangkok chicken sperm motility is lowest that other chicken breed. And the highest sperm motility is Lingnan chicken. This is in accordance with Purwanti (2009), the result of the research shows that Lingnan chicken has the biggest value in the egg fertility compare to the Arabian and Kedu chicken. The big amount of semen value in Lingnan chicken is expected that it has ability to bring the characteristic of egg fertility to its breeds.

V. CONCLUSION

From the result of study it was concluded that dilution treatments with higher percentage of egg yolk and longer storage cannot maintain the quality of semen because value of sperm motility rapidly decreased. Lingnan chicken has the highest sperm motility, this presumably that Lingnan chicken has the biggest value in the egg fertility compare to the Arabian and Kedu chicken. The big amount value of semen in Lingnan chicken is expected that it has ability to bring the characteristic of egg fertility to its breeds.

Suggestion and Future Work

Suggestions for future research should be no additional interaction between the egg yolks with the other diluents which may be possible to maintain sperm motility during storage. And storage the material should be on optimum temperature for storage of semen.

REFERENCES

- [1]. Budipurwanto, T. 2001. Studi Tentang Fenotip Ayam Buras Berdasarkan Sifat Kuantitatif dan Kualitatif. Fakultas Peternakan Universitas Diponegoro, Semarang. (Tesis)
- [2]. Blackly, J. And D.H Bade. 1994. Animal Science. Diterjemahkan Oleh Bambang Srigandono. Gajah MadaUniversity Press, Yogyakarta.
- [3]. Haryanto. 1996. Pengaruh Semen Ayam Segar Maupun Setelah Diencerkan dan Disimpan Melalui Inseminasi Buatan terhadap Fertilitas dan Kematian EmbrioTelur Ayam Kampung. J. Ilmiah Ilmu-Ilmu Peternakan. 3 (4) : 47-56.
- [4]. Hafez, E.S.E. (1974). Reproduction in farm animals. (3rd Ed). Lea and Fibiger, New York.
- [5]. Isnaini, N. 2000. Kualitas Semen Ayam Arab dalamPengencer NaCl Fisiologis dan Ringer's pada Suhu Kamar. J. Habitat. 11 (113) : 233-237.
- [6]. Nataamijaya, A. G., A. R. Setioko, B. Brahmantiyo, and K. Diwyanto. 2003. Performans dan Karakteristik Tiga Galur Ayam Lokal (Pelung, Arab dan Sentul). Prosiding Seminar Nasional Teknologi Pertanian dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan, Bogor.
- [7]. Negoro, F.P. 2011. Pengaruh Bahan Pengencer Tris Kuning Telur, Tris Susu Skim Dan Tris Susu Sapi Segar terhadap Kualitas Semen Sapi Pesisir dan Sapi Peranakan Ongole (PO).
- [8]. Purwanti, S. 2009. Pengaruh Penggunaan Berbagai Macam Pengenceran terhadap Motilitas, pH dan Daya Hidup Spermatozoa Selama Proses Pembuatan Semen Beku Ayam Kampung. Universitas Diponegoro, Semarang.
- [9]. Sarwono, B. 1995. Ragam Ayam Piaraan. Penebar Swadaya, Jakarta.
- [10]. Situmorang, P. 1991 Comparative growth performance, semen quality and draught capacity of the Indonesian swamp buffalo and its crosses. ACIAR Proc. (34). p. 102-112.
- [11]. Toelihere, M. R. 1979. Inseminasi Buatan pada Ternak. Angkasa, Bandung.