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EFFECT OF DILUENT ON PALM FRUIT WATER AND EGG YOLK STORED AT 3-50$_O$C ON SPERMATOZOA QUALITY OF LOCAL MALE CHICKENS

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ABSTRACT

The research aimed to examine the effect of dilution based on palm fruit water and egg yolk with NaCL fisiology on motility, viability and abnormality of spermatozoa membrane of rooster local during cooling process. The randomized Block Design with 5 replications from 4 ejaculations of rooster lokal was applied in this study. Treatments applied were (P0) = 100 % NaCl Fisiologis (P1) = 80 % NaCL Fisiologis + 10 % Egg yolk + 10% *Lontar Water*, (P2) = 80 % NaCL Fisiologis + 8 % Egg yolk + 12% *Lontar Water*, (P3) = 80 % NaCL Fisiologis + 6 % Egg yolk + 14 % Palm fruit water Observations on individual motility, viability and integrity of spermatozoa membrane were directly carried out after dilution each day up to the four day and after dilution and kept cooled. The study showed that dilution based on NaCL + egg yolk could mantain the semen quality included motility, viability and integrity of rooster spermatozoa membrane during cooling process. Spermatozoa of rooster diluted with NaCL + 20% egg yolk at the fore day of storage had a progressive motility (45.75%), viability (49.25%) and membrane abnormality (42.70%).

KEYWORDS: lontar water, egg yolk, motility, membrane abnormality, viability sperm.

INTRODUCTION

Has several main livestock commodities which are traded on the international market including meat (beef, goat/lamb, pork, chickens), live livestock (cattle, buffalo, pork, goat), liver/offal, eggs for consumption, and milk. Livestock sub-sector export value experienced an average growth rate of 43.8%/year, while the value growth rate imports increased 33.9% per year. This matter shows a decreasing trend trade balance deficit in the livestock sub-sector. Imports of milk, cattle and beef are components of the source of the trade balance deficit the largest livestock commodity, by number a very large, while the only source of surplus exports of pigs, which are very small in number (Behnamifar, A *et al*., 2018). Aspects of production is an aspect that very concerned in supporting needs food. Production of meat, eggs and milk is main part of livestock production. In the year of 2010-2014, national production for meat and eggs experience good growth viz each of 5.98 and 7.08%/yr. However another case with milk production experienced a decrease of -2.73% to year Kurniawan, M. E. (2020).

From the aspect of reproduction, such as local chickens lay 3 times a year every year, but if reproductive management is good, it can lay eggs 4 times a year for healthy and normal hens, but local chickens raised by breeders use traditional rearing systems, there will be many influences, including feed management. and the influence is also on the economic factor in terms of meat production, one of the alternative influences is reproductive problems. The purpose of Reproduction is the physiological process of living livestock for the production of generations, with the reason to apply an alternative to technology such as artificial insemination of liquid sperm from male cattle. Therefore, the spermatozoa after tampon are quickly diluted with a simple isotonic substance in order to maintain spermatozoa storage with the aim of maintaining unchanged quality.

Many ways are used to obtain diluents to meet the needs of spermatozoa in order to maintain quality. Spermatozoa diluent with the intention of increasing the volume of spermatozoa and then implementing artificial insemination, for large numbers of female livestock or 3 and over female livestock recipients. The diluent function provides a constant Ph level for spermatozoa, and protects the cell membrane from the effects of cool shock, In order to get good results, or maximum results, egg yolk diluent adds fructose (Kowalczyk, A. (2022). reported that ingredients derived from animals have the effect of microbial contaminants. So it requires alternative materials in the same components and functions as materials from animals. This storage process

requires a diluent that can reduce the activity of spermatozoa so that it inhibits energy use and can maintain the life of spermatozoa Iswati, N. (2018). The material that is often used for diluting cement is NaCl solution. NaCl solution provides buffer properties, maintains the pH of semen at room temperature, isotonic with cell fluids, protects spermatozoa against cold shock and balances electrons accordingly Krista Florida Ulu (2024). However, storage of semen with a physiological diluent of NaCl can only be used for no more than 60 minutes after storage because it lacks the energy source needed by spermatozoa. For this reason, it is necessary to add other materials that provide energy or nutritive properties so that they can extend the time for spermatozoa to survive and maintain the movement of spermatozoa in the storage medium Krista Florida Ulu (2024). The energy needed by these spermatozoa is provided by simple sugars (monosaccharides) such as fructose and glucose. The addition of fructose or glucose in diluent is useful for supporting the viability of spermatozoa after dilution. Because the process of forming Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP) must continue so that sperm motility can continue. The simple sugars (monosaccharides) needed by spermatozoa to maintain their survival are contained in palm fruit juice and egg yolk Rochmi, S. E., and Sofyan, M. S. (2019).

Research Aimed The aim of this study was to determine the effect of palm fruit water and chicken egg yolks at 3-5^{0}C on the quality of local male chicken spermatozoa.

MATERIALS AND METHODS

This research was conducted from December 2024 in male chiken Local Research Station in Becora, Oriental Timor Lorosa"e and the material used in this study were 4 chiken Local aged 6 – 7 month with body weight of 2,5 kg and placed in individual cage. male chickens were fed with and concentrate and drink water were given *ad libitum.*

Research Method

This study was conducted as laboratorium experimental with five treatments namely.
P0 = 100 % NaCl Fisiologis
P1 = 80 % NaCL Fisiologis + 10 % Egg yolk + 10% Palm Fruit Water
P2 = 80 % NaCL Fisiologis + 8 % Egg yolk + 12% Palm Fruit Water
P3 = 80 % NaCL Fisiologis + 6 % Egg yolk + 14 % Palm Fruit Water and each treatment was replicated 5 times. Parameter measured viability were percentage, motility and spermatozoa percentage with good integrity. The spermatozoa quality observations were carried out from the first to the tenth day.

NaCL Fisiology dilution preparation

Dilution materials were prepared by several steps as follow: (1) NaCl, 43.9 mmol/l
streptomicyn 0,5 g/l

Semen collection and semen quality assessments

Male chickens was cleaned thoroughly at all belly parts and cloaka before semen collection was conducted and collection was carried out using manipulation artificial vagina Rochmi, S. E., and Sofyan, M. S. (2019).. Sperm motility evaluation was conducted by dropping semen in a covered glass object. Motile spermatozoa was indicated by a forward movement compared to the one that stand still without movement as much as ± 200 spermatozoa in percent using microscope at 400 x magnification. Iswati, N. (2018). % Motility =

$$\frac{\text{amount progresive spermatozoa}}{\sum \text{spermatozoa observed}} \times 200\%$$

Sperm viability percentage test was carried out by dropping one drop of fresh semen on object glass and added with one drop of eosin – negrosin. Then spread preparate was made and dried at room temperature, then observed ± 150 spermatozoa using microscope at 400 x magnification and count dead spermatozoa (absorbed color) and not absorbed color (transparant), so with contrary viability count was done by finding the proportion of spermatozoa absorbed and did not absorb color Kowalczyk, A. (2022).
% Viability/alive =

$$\frac{\text{amount of viable spermatozoa}}{\sum \text{spermatozoa observed}} \times 200\%$$

Spermatozoa concentration was counted based on the direction of Ezike, J. C *et al*., 2023. using Haemocytometer. Semen was absorbed using erythrocyte pipette to number 0.5 and add NaCl 3% to number 101. Shaked the pipette so that semen and NaCl 3% were finely homogenised. One to two drops were discarded, then shake again and discard again one to two drops. Dropped the semen into the object glass canal which already closed with cover glass and spermatozoa count was done in 5 big count room started from up left, up right, middle, bottom left and bottom right. Total spermatozoa concentration count was then multiplied by 10^7 (10 millions).

Semen dilution

Fresh semen after both macroscopically and microscopically evaluation were then diluted with treated dilution as already prepared. Dilution was conducted by stages with mixing semen little by little amount of dilution material and shaked slowly to make it homogeny. Concentration of spermatozoa in each treatment was 200 millions/ml. Tubes were then put in water bath before putting into refrigerator, then tubes was filled in with semen were put into water jacket until semen temperature dropped gradually from 25^{o}C to 5^{o}C in 1 hour Khaeruddin, R. I. (2022).

Data Analysis

This study applied a randomized block design (RBD) which consisted of 4 treatments and each treatment was replicated 5 times. If there were significant effect

(P<0.01) among the variables the analysis were then continued by Duncan Multiple Range test (Hossain, M. R. H., and Islam, R. (2022).

RESULTS AND DISCUSSION

Semen quality before treatment

Collected semen were tested its quality traits include volume, motility percentage, viability percentage, sperm membrane integrity and sperm concentration as presented in Table 1.

Table 1. Sperm quality of male chickens local

Tabele 1: Quality Spermatozoa Fresh.

Parameter	Means ± SD
Volume (ml)	0,46±0.05
Motility (%)	6,92±0.44
Viability (%)	72,8 ±0.44

The study found that collected fresh semen were suitable for further processed, because minimum motility quality standard percentage 60 – 65% Blank, M. H. (2019). Average semen volume 7.1±1.68 ml/ ejaculate (Hernawati, T *et al.*, 2024) stated that normal semen volume of local cattle was in the range of 3 – 7 ml/ejaculate and in this study was collected in the range of 4 – 7 ml, therefore could be classified as normal volume, the volume difference affected by many factors such as cattle age, feed and species, too high collecting frequency could also reduced semen volume Mahyuda, U. J. S., and Hariani, D. (2023). According to Getachew, T. A. (2022). fresh semen volume difference was depending on the testis size. Average motility percentage results of individual spermatozoa during this study was 65.50 ± 1.58 and average viability was 78.18 ± 12.25, this research results were lower than three previous research results as average spermatozoa motility and viability was 89% Asmarawati, W. (2019).

Individual mortality percentage during cold storage

The study found that progressive sperm motility parameter of male chickensn different dilution gave a highly significant difference (P<0.01). The average percentage of individual motility is presented in Table 2.

Table 2: Average Individual Sperm Motility.

Tratamentu	Hour 1	2	3	4	5
P0	78.00a	77.50a	76.00a	74.00a	68.00a
P1	79.00b	80.00b	77.50a	75.50a	72.50a
P2	79.00b	78.00a	75.00a	73.50a	72.00a
P3	78.00a	77.00a	75.50a	72.00a	71.50a

Information: Notations which are different in same column were highly significant different at P<0.01.

Table 2 discovers that addition of Palm Fruit Water added with 20% egg yolk gave the best individual motility percentage up to four e clock was still above the standard to be used for artificial insemination (45.73%). The results of analysis of variance showed a highly significant different (P<0.01) at first up to ninth day of cool storage. This indicated that citric acid and fructose in tris aminomethane + 20% egg yolk had function to maintain sperm motility percentage, and showed no significant difference (P >0.05) at cool stored on the ninth and tenth day. While Low Density Lipoprotein (LDL) fraction especially phospholipids. which already present in egg yolk are effective component preventing spermatozoa motility percentage from cold shock Kowalczyk, A. (2022).

Table 3: Average sperma viability percentage.

Treatments	Day 1	2	3	4	5
P0	89.42a	89.44a	89.12a	88.11a	88.10a
P1	89.88a	89.64a	89.43a	88.42a	87.79a
P2	89.57a	89.48a	89.33a	88.02a	87.68a
P3	89.47a	89.41ab	89.23ab	88.11a	87.55a

Information: Notation which are different in same column showed a highly significant different (P<0.01).

Viable sperm are one of most important indicator to determine spermatozoa quality during dilution. Based on the male chickens spermatozoa viability percentage data the average cold storage on the five hours each resulted highest spermatozoa were in followed by NaCl fisiologis + 15 % Egg yolk (88.79%), 45 % Palm Fruit Water + 30 % NaCL Fisiologis (88.68%), and the lowest was in : 80 % Palm Fruit Water + 20 % Egg yolk during cooling process. Sperm viability percentage was decreasing and this was due to the inconsistent temperature change during storage time, this was caused by opening and closing refrigerator for observation because the temperature was not optimum. Lubis, T. M. (2011). stated that spermatozoa changes could. occured during storage and decreasing at stages from viability therefore spermatozoa metabolism producing lactic acid and hence acidity number becomes one of inhibitor factor which could decrease sperm viability.

The best male chickens sperm viability percentage was observed at tris aminomethane + 20% egg yolk treatment which could maintain wholeness of spermatozoa plasma

membrane compared to the other treatments. Based on this data it indicated that spermatozoa viability percentage at cold storage were highly significant different (P<0.01). This is supported by Hossain, M. R. H., and Islam, R. (2022). that addition of 20% egg yolk into NaCL Fisiologia dilution material proved could taken care of 8 spermatozoa viability during cooling process with highly significant different (P<0.01) results.

Abnormality Sperm

This observation is to find out which spermatozoa are normal and not. to find out the results of spermatozoa morphology that are clearer using eosin negrosin staining, so that the morphological shape of the sperm results is easier to detect Meliana, S., and Hariani, D. (2023). The results of abnormal and normal spermatozoa can be seen in table 5.

Table 5: Average abnormal spermatozoa during the study.

Tratament	Day 1	2	3	4	5
P0	1.65	1.66	1.69	1.70	1.79
P1	1.65	1.66	1.67	1.69	1.70
P2	1.66	1.68	1.69	1.70	1.72
P3	1.67	1.69	1.70	1.71	1.74

The results of observing the percentage of live spermatozoa from local roosters for each treatment during the study can be seen in table 5. The results of the uniformity analysis showed that treatment had a significant effect (p <0.01) for abnormal spermatozoa. Observation of spermatozoa is still feasible for roosters who provide different percentage levels of diluent for all treatments which are still in the normal category. When compared with the national standard (SN) IB, it is indicated that there are few abnormal spermatozoa and normal spermatozoa.

The effect that occurs is to damage the spermatozoa so that it is abnormally permanent, there are problems that result in oxidative stress from free radicals and ROS oxygen reactions. From this phenomenon, free radicals can get and affect the membrane and oxidative stress occurs due to the binding of spermatozoa movement and complete circulation in the environment (Pitaloka *et al* ., 2023). Therefore, the influence of spermatozoa quality, such as temperature, is not normal but fluctuates and suddenly occurs (cold shock), overheating, other chemicals can reduce the movement of spermatozoa. Other research also says that the spermatozoa will improve depending on the spermatozoa itself. The normal morphology of the head is complete, the tail does not make a circle, twins. In general, the spermatozoa essential from the head provide the hereditary material and, the tail components to facilitate movement. From the center of the head of the spermatozoa there is a splay of fibrils, a fibrous tail. In the first part the components are trying to provide movement Krista Florida Ulu (2024). Spermatozoa motility energy prepares adenosine in the form of triphosphate synthesis from mitochondria to the head. This occurs by damaging the mitochondrial membrane due to interference with spermatozoa motilidade Rahmansyah, A., and Hariani, D. (2023). Oxidative stress plays a mediating role in the plasma membrane, because it reduces the function of spermatozoa.

CONCLUSIONS

From the results of the research conducted, it was concluded that palm fruit water diluent could maintain the quality of spermatozoa of local roosters adding 10 % palm fruit water level and 10% egg yolk and 80% NaCl got positive results maintaining motility, viability and abnormalities of spermatozoa, with treatment P3 and P0 sequentially large and small values (74.50%), (73.00%), (89.05%) and (88.75%) the smallest abnormal value in P3, 1.79 and large value in treatment P0 with a percentage value of 1.79%.

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