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Effect of the Addition of Fresh Moringa Leaf Extract in Egg Yolk Dilution and Physiological Sodium Chloride on the Quality of Spermatozoa of Landrace Pigs

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ABSTRACT: This study aims to determine the effect of adding fresh moringa leaf extract to egg yolk diluent and NACl on the spermatozoa quality of landrace pigs. This study is experimental research using the Complete Random Design (RAL) method consisting of 5 treatments and 5 replicates. The treatment used in this study is: P0= NACl Fis 2,4% KT, P1= NACl Fis 2,4%+KT 0,6 EDKS 1%, P2= NACl Fis 2,4%+KT 0,6 EDKS 2%, P3= NACl Fis 2,4%+KT 0,6 EDKS 3%, P4= NACl Fis 2,4%+KT 0,6 EDKS 4%. The entire treatment is stored in *coolboox* at a temperature of 18-20°C. Evaluation of spermatozoa motility, viability, abnormalities and survival is carried out every 8 hours. The results of the study showed that the 24th storage hour with P1 (46.00±2.23), P2 (47.00±6.70), P3 (48.00±12.04) and P4 (49.00±15.16) treatment had motility above 40% that was feasible for IB. . It was concluded that the addition of fresh moringa leaf extract to the egg yolk and NACl could maintain the quality of landrace pig spermatozoa even at the highest level (40%).

KEYWORDS: Fresh moringa leaf extract, egg yolk, NACl, landrace pig

INTRODUCTION

Government programs in an effort to improve the genetic quality of livestock and the number of pig livestock (IB) populations, among others, through the implementation of artificial insemination programs, the success rate of IB is influenced by the main factors, namely the quality of semen of female cattle as an accessor, inseminator skills, and knowledge of breeders. These four factors are related to each other and if one is low, the IB result is also low (Tambing, 2001). To maintain the quality of semen, there are several things that need to be considered in storing fresh semen so that the vitality of spermatozoa does not decline quickly, namely avoiding excessive heat with air exposed to direct sunlight for too long and avoiding excessive shocks (Tolihere, 1993). The vitality of spermatozoa outside the body is very low and easily die (Partodihardjo, 1992). Pig livestock have a different cement from other ruminant semen such as cattle and goats, because pig semen spermatozoa have a plasma membrane composition, namely *phosphatidylethanolamine* and *sphingomyelin* reaching 24% and 14%, so it is easy to experience *cold shock* during the preservation process (Paulenz *et al*., 2000). Pig cement can last at a temperature of 15-20 ℃ and the storage capacity of pig cement is relatively short, which is 3 -7 days depending on the diluting material used (Paulenz *et al*., 2000; Gadea, 2003; Robert, 2006).

The selection of diluting materials is very important to be considered to improve the quality of landrace pig spermatozoa Egg yolk dilution has been widely used as a cement diluent because it contains *a buffer* that acts as a buffer in maintaining, viability and fertility of spermatozoa (Garner dan Hafes, 2000). The main composition of egg yolks is composed of water, proteins, fats, carbohydrates, minerals, vitamins and contains all kinds of essential amino acids in considerable quantities (Sarwono, 1995). Egg yolks also contain lipoproteins and slychees can protect the integrity of the lipoprotein sheath of spermatozoa cells and prevent *cold sores* (Gamer dan Hafes, 2000). Moringa leaves are widely known in Indonesia, especially in rural areas, but have not been utilized optimally in life. Moringa trees are widely planted as living hedges, plants along fields or rice paddies, serving as greening plants. In addition, the moringa plant is also known as medicinal planting with all parts of the moringa plant starting from the leaves, skin, seeds, to the roots (Simbolan *et al*., 2007). Moringa leaves contain nutrients such as carbohydrates, proteins (essential and non-essential amino acids), fats, vitamins (which are soluble in water and fat), minerals and the amount is much more in the form of leaf powder than in the form of pods and fresh leaves (Bey, 2010). Carbohydrates, proteins and fats can be used as a source of mineral energy to maintain the harmony between acid-base and fluid balance in the body (*buffer*), vitamins that play a role in metabolic processes and life maintenance as well as antioxidants to protect cement from microbial contamination (Tilman, 1984).

Moringa leaves contain 46 antioxidants including Vitamin A, Vitamin C, and Vitamin E. Moringa leaves (*moringa oleifare lam*) also contain Vitamin K, B Vitamins, alanine, alpha-carotene, arginine, beta-carotene, beta-sitosteral, caffeine-oylquinic acid, capesterol, carotenoids, delta-7-avenasterol, *glutathine, histidine, indole acetic acid, indoacettonitrile, kaempferal leucine, lutein,*

methionine, myritic acid, palmitic acid, prolamine, proline, quercetin, rutin, selenium, threonine, tryptophan, xanthin, xantophicon, zeatin, zeasantin, zinc (kurniasih, 2013). Oka *et al.* (2016) states that moringa leaves (*moringa oleifera lam*) have an antioxidant content of 114.51 mg/L and are rich in *phytochemicals*, proteins, carotene, vitamin C, minerals, amino acids, flavonoid and phenolic compounds. Moringa leaf extract (*moringa oleifera lam.*) can improve spermatozoa quality and testes shape in male rabbits (ikpechukwu at al., 2013). Moringa *leaves (Moringa oleifera lam*) contain high anti-oxidants and some bioactive compounds of the flavomoite group such as *quercetin, kaempferol and proanthocyanidin* (Fitria el al., 2013). In addition to moringa leaves, physiological Sodium Chloride (NaCl) can also be used as a cement diluent because it can maintain spermatozoa motility outside the body of livestock up to 12 hours after storage (Tanaka dkk., 1994). The addition of thinners aims to extend the life span of spermatozoa. At room temperature, fresh spermatozoa can live for about 2 hours, but with the addition of diluent spermatozoa can live for 6-24 hours at *refrigerator temperature.*

RESEARCH METHODS

Time and location of the research

This research was carried out at the Reproductive Laboratory, Faculty of Animal Husbandry, Marine and Fisheries University of Nusa Cendana Kupang, East Penfui Village, East Nusa Tenggara Province (NTT).

Research Materials and Tools

Research materials

The materials used in this study were fresh semen of male pigs collected from one male pig that was two years old and had reached sexual maturity in healthy condition, egg yolk and physiological NaCL used as a diluent, fresh moringa leaf extract, as well as penicillin and streptomycin which were used as antibiotics.

Research Tools

The tool used in this study is Prepare trained male pigs that will be cement shelter. Pen equipment Equipment for making cement thinner (cup cups), measuring cups, pipettes, filter paper, tweezers, tissues, funnels, spoits, stainless basins, stirrers, spin bars, and centrifuges). Dummy cement reservoir (artificial female, cement container tube and gauze).

Analytical balances, spatulas, alluminium foil, elenmeyer tubes, treatment tubes, pipettes, and measuring tubes. Cement evaluation equipment (microscope, glass object and glass cover, heating table, pH paper, hemacyometer, pipette, scaled tube, to calculate the concentration and conthing chamber complete with erythrocyte pipettes. Cool box as a cement and thermomet storage device.

Material

The ingredients used in this study were fresh semen of lanrace pigs, physiological sodium chloride, purebred chicken yolk and moringa leaf extract (EDKS), spermatozoa staining material (eosin-negrosin), Aquabidestilata and 70% alcohol, Antibiotics (*penicillin and streptomycin*).

Metode Penelitian

This type of research is an experimental research with a Complete Random Design (RAL) research method consisting of 5 treatments and 5 replicates. The treatments used in this study are:

P0 = Physiological NaCI 80% + egg yolk 0.6%

P1 = Physiological NaCI 80% + Egg Yolk 0.6% + Fresh Moringa Leaves 1% P2 = Physiological NaCI 80% + Egg Yolk 0.6% + Fresh Moringa Leaves 2% P3 = NaCI Physiology 80% + Egg Yolk 0.6% + Fresh Moringa Leaves 3% P4 = Physiological NaCI 80% + Egg Yolk 0.6% + Fresh Moringa Leaves 4%

Preparation of egg yolks

Before the eggs are used, the egg shells are cleaned using 70% alcohol cotton. After that, break the egg at its pointed part or corner. Pour in all the egg whites and separate from the yolks. The yolk that is still wrapped in the vitellar membrane is placed on a filter paper, then tilted and moved in circles so that all the egg whites can be absorbed completely. Break the vitelin membrane, put the yolk in a measuring glass and ready to be used as needed.

Preparation of NaClF-KT Diluent

In the process of making NaCL, it is weighed using an analytical balance. And adding aquades to the measuring dock, and homogenizing it to the point of terah, then there is a reaction marked by a fixed clear color and there are bubbles, and marked by the solution being cold. The physiological NaCl solution contained in the package is mixed with egg yolks until homogeneous with a ratio of 4:1. The mixed NaCL solution was divided into five tubes according to the number of treatments, and each tube was added with 2.4% lactose sequentially for the P0, P1, P2, P3, P4 treatments. Add to the five tubes containing the diluent 1,000 IU of penicillin and 1,000 IU/mL of threptomycin; and then mixed until homogeneous.

Stages of Making Moringa Leaf Extract

The fresh moringa leaf extract used in the study was fresh moringa leaves (*Moringa Oleifera L am*). The leaves are picked from the stalks and washed then blended without water and filtered using filter paper 1 time. Moringa leaf extract that has been filtered,

measured to the same degree as the pH paper indicator. If the pH is still acidic, it is added with egg yolks until the pH reaches neutral or equal to the pH of pig semen.

Penampungan Semen

The livestock used as a source of semen in this study is one landrace male pig, which is in good health, a proportional body has normal reproductive organs and has been trained to accommodate the semen. The process of collecting cement in pigs is carried out using the massaging method using a clean ladder and using the hend glove method. The semen that has been obtained is then evaluated both macroscopically (volume, color, pH, and semen concentration) and microscopic evaluation of semen (spermatozoa's motility, viability, abnormality, and survival)

Evaluation Stage

Evaluation of cement quality can be carried out as soon as the cement is stored, namely a macroscopic examination which includes checking volume, color, consistency and pH. Microscopic examination includes (mass movement as well as individual), concentration, viability and abnormalities of spermatozoa.

The source of cement came from a male pig of the Landrace nation, in a healthy condition of two years old, and having a volume of 148 mL. Penampungan semen dilakukan dua kali seminggu, dengan metode *massage*.

Cement evaluation

Cement evaluation can be done in 2 ways, namely microscopic and macroscopic.

Macroscopic. Macroscopically it includes the assessment of:

Volume

The volume of semen is highly dependent on the species, age and health level of the female or angler's tiser. The volume of cement can be known directly by looking at the cement collection tube.

Warna.

The assessment of the color of the cement can be seen from the cement that has been accommodated in the cement container tube.

Clear white : less or not good

Milk beige : Good Gray beige : Very Good yellow (10%): Normal

Red leads brownish: Blood (Fresh-long) Yellowish-green brass: Pus (Active)

Degree of Acidity (pH)

The degree of pH acidity of cement can be determined using keratslakmus, by dripping cement on litmus paper, The color change on the litmus paper is matched with the color of the pH indicator paper. Normal pH ranges from 6.2-7.5(6.8-7.0).

Construction

The typical smell of pig semen and its consistency depends on the amount of ejaculation and the method of storage used.

Viscosity

The semen obtained using the artificial vaginal container method is somewhat thick compared to the electroejaculator method. The semen obtained from the results of the first and second ejaculation looks thicker than the third ejaculation and so on.

pH cement

The pH measurement of cement can be measured using pH paper, to obtain accurate data, perform calibration before the tool is used. The normal pH of cement is around 6.4 to 7.0.

Microscopy

Microscopic sperm quality evaluation is carried out using a microscope which includes:

Motilitas

The percentage of spermatozoa motility was determined by observing the spermatozoa under a microscope with 10x40 objective lens magnification. The assessment of the percentage that moves progressively is determined subjectively on different values and ranges from 0% to 100% on a scale of 5%. Spermatozoa Concentration The calculation of the percentage of spermatozoa uses a neubauer calculation, namely by sucking the eosin in the tube using an ehrythrocyte pipette of 0.5 ml. Then the 0.2 suction eosin to the 1.01 mark is then carefully homogenized for 2-3 minutes by forming a number eight. Drop the first few drops on the tissue and then drop them in the gap of the neubauer counting room and cover it with a glass cover. Make observations under a microscope by zooming in 10x40. Then count the number of spermatozoa cells in the five chambers in a diagonal direction. In the 5 counting chambers there are 16 small rooms with a volume with each small room 0.1 mm 3 and dilution 200 times.

Calculation using hemochito meter X = the number of spermatozoa obtained. X x sperm volume x 106 sperm/mL.

Rating: best: ≥1000x106

cells/ml medium: 800-1000x106cells/mL.

Poor: < 800x106cells/mL.

Viability

The viability of spermatozoa is known through microscopic observation at a magnification of 10x40. The measurement was made after the spermatozoa were stained with eosin negrosin. Live spermatozoa are clear or white, while dead spermatozoa are purplish-

red. The calculation of spermatozoa viability is obtained according to the formula:

$$\text{Viability} = \frac{\text{number of live spermatozoa}}{\text{total of spermatozoa}} \text{X}100$$

Abnormalitas

Abnormalities based on the number of spermatozoa that have an abnormal shape using the formula:

$$\text{Abnormalitas} = \frac{\text{number of spermatozoa}}{\text{total of spermatozoa}} \text{X}100$$

Research variables

The parameters observed in this study are motility, viability, abnormality and life expectancy.

Motilitas spermatozoa

Spermatozoa motility is the ability of spermatozoa to perform forward or progressive movements. The purpose of motility calculations in spermatozoa is to observe living and dead spermatozoa. Spermatozoa that are not progressive and stationary in place can be categorized as dead spermatozoa, while progressively moving ones indicate living spermatozoa.

Spermatozoa viability

The viability of spermatozoa is influenced by the need for nutrients for sperm. The nutrients used by spermatozoa will be used as energy, so that if the nutritional needs of spermatozoa decrease, it will result in a decrease in spermatozoa viability (Hidayahtrrahmah, 2007). The viability of spermatozoa is characterized by the length of time the spermatozoa survive.

Spermatozoa Abnormalities

Spermatozoa abnormalities can be differentiated into primary abnormalities and secondary abnormalities. The determination of spermatozoa abnormalities is the comparison between abnormal spermatozoa and normal spermatozoa (Fitrik dan Supartini,2012).

Durability

Life expectancy is characterized by the presence of motility and mobility which is used as a benchmark or an easy and simple way in assessing semen for IB, the best individual movement is forward movement and the percentage of pig semen motility below 40% shows a poor semen value and is often associated with infertility.(Toelihere, 1981).

Data Analysis

Data analysis began with calculating the average and standard deviation then continued with the Analysis of Variance (ANOVA) test using SPSS.20 and if there was a difference, the further test was continued.

RESULTS AND DISCUSSION

Characteristics of Landrace Pig Fresh Cement

The evaluation of fresh semen in this study was carried out immediately after the cement storage was determined in two ways, namely through macroscopic and microscopic assessments. The results of the examination of fresh cement in one reservoir are very important to determine the quality of the cement which is then used as an indicator for whether or not the cement can continue in the dilution process.

Based on the macroscopic and microscopic evaluation of landrace fresh pig semen, the results are shown in Table 2.

Tabel. 2. Kerakteristik semen segar babi landrace

Characteristics of cement	Observation results
Volume (mL)	160±9,55
Warna	White Susuh
Konsisten/ kekentalan	Encer
Ph	6,94±0,13
Individual motion / Motility (%)	83,00±2,74
Concentration (x106/mL)	91,00±4,30
Viability/Life (%)	2,40±0,55
Abnormal (%)	217±9,82

Table 2 shows the results of the characteristics of fresh semen from ejaculation that were evaluated macroscopically, the volume of fresh semen was obtained with an average of 160±9.55 ml, the volume of fresh semen obtained in this study was the same as the previous study which stated that the volume of fresh semen ranged from 100-150 million cells/mL (Garner dan Hadfez 2000) and lower compared to the study of Toelihere *et al.* (2014) and processing the volume of landrace pigs reached 160±9.55 mL, namely. In general, the normal characteristic of pig fresh semen according to Robert (2006) is that the volume of pigs without gelatin ranges from 100-150 mL. Toelihere (1993), The volume of cement is influenced by the type of livestock, nation, age, body size, feed

quality, frequency of shelter, level of livestock health, production status, and the level of restraint (*false mouth*) during the shelter process.The color of the semen from the study was milky white with a dilute consistency with a physiological pH of 604±0.13 and had the same pH range as the results of the research of Garner and Hafez (2000) which was 6.4-7.8. The results of the study obtained a low cement pH from the research of Sumardani (2007) which was 7.7±0.44 and Gadea (2003) which was 7.4±0.2. Influencing factors are age, stimulus level, ejaculation frequency, environment and feed quality (Johnsonet al., 2000 and Feradis 2010).

The observation of spermatozoa motility of landrace pigs in this study obtained a percentage of 83% and is still stated to be good, the results of this study are almost the same as the observations of Shukla *et al.,* (1992) which obtained 50-80%, and the results of observations of Garner and Hafez (2000) in cattle which range from 40-75%. The high motility value is also caused by the diluting material used (EDKS) and the number of supporting factors, namely genetics, age, light, temperature, maintenance management, storage frequency and environment (Everett dan Beans 1982).

Effect of Treatment on Spermatozoa Motility

The motility or mobility of spermatozoa is one of the parameters used in the analysis of semen quality. Motility is essential for spermatozoa to pass through the *cervix uterine* and *uterotubal junctions,* and even more important for the penetration of cumulus cells and the *lulucidal zone of the egg.* Therefore, motility estimation is very important in controlling semen quality. Average motility value of landrace pig spermatozoa from each treatment

Table 3. Effect of treatment on spermatozoa motility (%)

Hours to-	Treatment					P-v
	P0	P1	P2	P3	P4	
0	84,00±2,23^{a}	84,00±2,23^{a}	84,00±2,23^{a}	84,00±2,23^{a}	84,00±2,23^{a}	1,000
8	48,00±4,47^{a}	64,00±5,47^{b}	63,00±2,73^{b}	62,00±10,95^{b}	64,00±12,94^{b}	0,028
16	39,00±2,23^{a}	56,00±6,51^{b}	57,00±2,73^{b}	55,00±11,72^{b}	56,00±14,74^{b}	0,024
24	35,00±3,53^{a}	46,00±2,23^{ab}	47,00±6,70^{ab}	48,00±12,04^{ab}	49,00±15,16^{b}	0,158
32	26,00±4,18^{a}	28,00±9,08^{a}	27,00±12,04^{a}	24,00±14,74^{a}	19,00±10,83^{a}	0,999

a,bDifferent superscripts on the same line show noticeable differences(P<0.05). Ket. P0 : No addition of EDKS, P1 : Addition of 1% EDKS, P2 : Addition of 2% EDKS, P3 : Addition of 3% EDKS, P4 : Addition of 4% EDKS.

Based on Table 3 above, it shows that the presentation of spermatozoa motility after dilution at 0 hours does not differ significantly between each treatment (P > 0.05). However, at the observation of the 8th to the 32nd hour, the percentage of motility between treatments occurred a decrease in semen. If viewed from the percentage of motility above, at the 24th hour of observation the P0 treatment was lower than the P1, P2, P3 and P4 treatments. Low P0 treatment due to the nutrients contained in moringa leaf extract greatly affects spermatozoa motility. At the 24th hour of observation, p4 treatment (4%) spermatozoa motility was able to survive above 40% and was suitable for IB. Meanwhile, at the 32nd observation hour there was a very drastic decrease, this was due to the decrease in energy contained in moringa leaf extract resulting in the number of spermatozoa that did not move forward. In general, the phenomenon of decreased spermatozoa motility after long storage is caused by a decrease in spermatozoa nutrients and the influence of toxic substances that are a byproduct of the spermatozoan metabolic process (Rizal *et al*., 2004).

The decrease is due to the possibility that the level of moringa leaf extract as supplementation in the diluent is too high. According to Fuglie (2001), the vitamin C content in moringa leaves is 17.3 mg/100 mg and vitamin B3 is 8.2/100 mg. The addition of vitamin C and vitamin B3 at cement retailers can cause changes in pH because vitamin C is acidic. Spermatozoa are very sensitive to changes in pH medium which can have an impact on the decrease in sperm quality (Rizal and Herdis, 2010). In addition, moringa leaves also contain anti-nutrient substances such as saponins and tannins, these substances can be toxic to spermatozoa at high doses. Thus, at high doses, moringa leaf extract will produce negative effects on sperm life during storage.

The right administration of moringa leaf extract provides maximum results to prevent lipid peroxidation on the plasma membrane of spermatozoa by preventing or breaking the reaction of lipid peroxidation chain on the plasma membrane of spermatozoa, Kurniasih (2013) stated that moringa leaves are rich in nutrients, with 46 types of moringa leaf antioxidant content. In addition to being used as foodstuffs, moringa leaves have been reported to be a rich source of macronutrients and micronutrients that also contain β kraten, protein, vitamin C, calcium, and potassium; and also acts as a natural source of antioxidants. These macronutrients and micronutrients are very useful for supporting sperm motility (Johnson *et al.,* 2000). Antioxidants as compounds that can delay, slow down and slow down the lipid oxidation process, by delaying or preventing the occurrence of free radical oxidation reactions in lipid oxidation (Shui *et al.,* 2004). So based on the motility percentage data after landrace pig liquid cement dilution, P0 treatment is suitable for artificial insemination from 0-16 hours, while for P1, P2, P3 and P4 it is suitable for artificial insemination from 0-24 hours. As based on Indonesian national standards, (2014) liquid semen after preservation must show a spermatozoa motility of at least 40% as the IB standard.

Effect of the Addition of Fresh Moringa Leaf Extract in Egg Yolk Dilution and Physiological Sodium Chloride on the Quality of Spermatozoa of Landrace Pigs

Effect of Treatment on Spermatozoa Viability

The viability of spermatozoa is determined by *the eosin-negrosin* staining method. Living spermatozoa are characterized by not absorbing dyestuffs, while dead spermatozoa are characterized by spermatozoa cells that absorb dyestuffs. The viability of landrace pig spermatozoa is shown in Table 4

Table 4. Effect of treatment on spermatozoa viability (%)

Hour			Treatment			P-v
	P0	P1	P2	P3	P4	
0	91,00±4,30[a]	91,00±4,30[a]	91,00±4,30[a]	91,00±4,30[a]	91,00±4,30[a]	1,000
8	71,92±7,03[a]	75,13±5,46[a]	73,15±3,79[a]	74,52±6,22[a]	71,82±7,12[a]	0,871
16	65,65±10,0[a]	67,65±6,83[a]	70,32±4,96[a]	67,15±7,89[a]	67,19±9,67[a]	0,924
24	58,08±5,79[a]	60,82±5,16[a]	60,88±8,49[a]	60,30±13,1[a]	59,48±13,6[a]	0,990
32	51,01±9,40[a]	52,85±7,7[a]	50,22±13,24[a]	53,98±14,4[a]	54,39±16,9[a]	0,979

a,bThe same superscript on the same line shows a noticeable difference (P<0.05). Ket. P0 : No addition of EDKS, P1 : Addition of 1% EDKS, P2 : Addition of 2% EDKS, P3 : Addition of 3% EDKS, P4 : Addition of 4% EDKS.

Table 4 shows that the results of the statistical test of spermatozoa viability after dilution for all treatments are not significantly different (P>0.05). So at the observation hour from the 8th to the 32nd observation hour, the percentage between the treatments did not differ significantly (P>0.05). At the 32nd hour of observation, P0 (SKT+2.4 NACL FLS), P1 (SKT+1% EDKS), and P2 (SKT+2% EDKS) treatment were not noticeable (P>0.05) and were able to maintain the viability of spermatozoa up to the 32nd hour with an average of P0 (51.01±9.40%), P1 (52.85±7.7%) and P2 (50.22±13.24%) and suitable for artificial insemination (IB) because it was able to survive up to a minimum of 20%. This shows that the addition of moringa leaf extract has no effect on the viability of spermatozoa.

Therefore, the results of this study show that the best viability presentation is P0 with a value of 51.01±9.40% compared to the P1 treatment of 52.85±7.7%, P2 50.22±13.24% and P3 53.98±14.4%. This suggests that the P0 treatment during storage gets the energy supply from the egg yolk tris to survive. Meanwhile, treatment with the addition of EDKS is not optimal in maintaining sperm quality so there is no effect on the viability of spermatozoa in pig lanrace. These results are based on the research of Hermawan *at al*. (2019) stated that When the viability of spermatozoa is usually higher than When EDKS is not added in treatment. The percentage of spermatozoa viability is an indicator to assess the quality of semen, the higher the percentage of semen viability, the better the quality of the semen (Rizal and Herdis, 2008). Living spermatozoa are characterized by being colorless, then the head of spermatozoa is by eosin, while spermatozoa that die their head will be purplish-red because they absorb eosine dyes. The value of the percentage of spermatozoa viability is usually higher than the percentage of spermatozoa motility. At the observation hour of 32, the percentage of landrace pig spermatozoa in moringa leaf extract dilution was around 50.22±13.24% and was suitable for IB because the viable viability for IB was above 45% (Sumerdani *et al*., 2008). The high percentage of spermatozoa viability is due to the fact that moringa leaf extract in the diluent can maintain spermatozoa viability by inhibiting the formation of pleated peroxide which causes damage to the spermatozoa membrane.

Effect of Treatment on Spermatozoa Abnormalities

Spermatozoa abnormality is a physical abnormality of spermatozoa that occurs due to genetic factors, environmental temperature, stress of preparation, and at the time of cement storage in (Arviantini *et al*, 2006*)*. The abnormal value of spermatozoa can be seen in table 5.

Table 5. Effect of treatment on spermatozoa abnormalities (%)

Hours			Perlakuan			P-v
to-	P0	P1	P2	P3	P4	
0	2,80±0,83[a]	2,80±0,83[a]	2,80±0,83[a]	2,80±0,83[a]	2,80±0,83[a]	1,000
8	6,80±2,16[a]	7,60±1,51[a]	7,00±1,58[a]	8,40±2,07[a]	9,20±1,78[a]	0,248
16	7,00±2,00[a]	6,80±1,48[a]	7,40±2,19[a]	7,00±2,91[a]	7,80±3,27[a]	0,969
24	7,80±2,38[a]	7,60±1,51[a]	7,20±1,92[a]	6,80±0,83[a]	7,40±1,81[a]	0,915
32	7,40±2,88[a]	6,80±2,94[a]	7,20±3,11[a]	7,20±3,89[a]	6,80±3,49[a]	0,998

a,b Different superscripts on the same line show noticeable differences(P<0.05). Ket. P0 : No addition of EDKS, P1 : Addition of

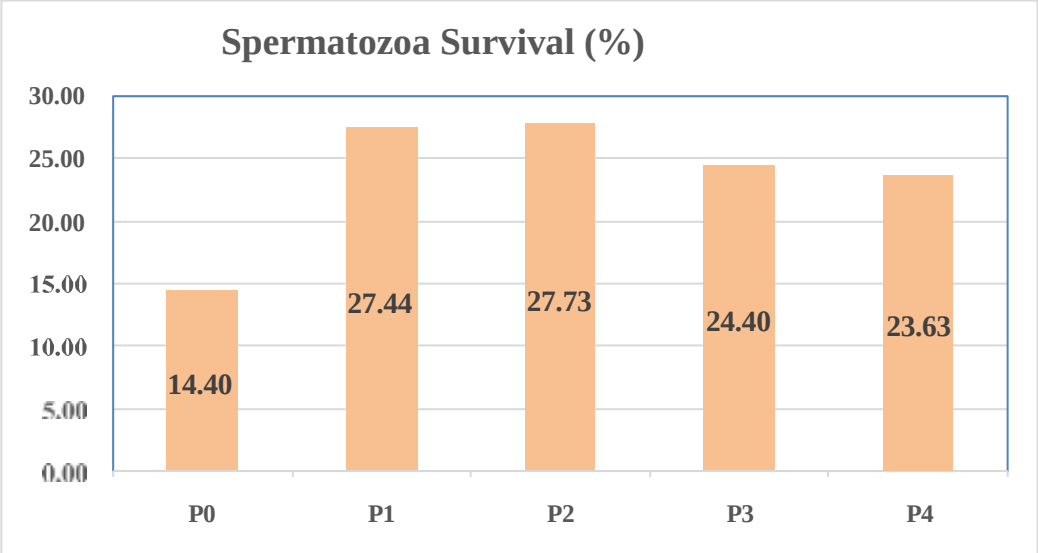
1% EDKS, P2 : Addition of 2% EDKS, P3 : Addition of 3% EDKS, P4 : Addition of 4% EDKS.

In the table above, the average percentage of spermatozoa abnormalities shows that at the observation hour 0 to the 32nd hour there is no real effect (P>0.05). This shows that the addition of moringa leaf extract does not affect the abnormality of landrace pig spermatozoa. The high level of spermatozoa abnormalities in P4 treatment may be due to the concentration of moringa leaf extract that is too high which can lead to excessive water excretion from within the sperm cells that continues during cell contraction. The general mean of abnormalities as a result of this study was (7.00±2.00). This is still required to be declared normal. Presentase abnormalities that are declared normal, which should not be more than 20%, this illustrates the bottom with the addition of moringa leaf extract is more optimal in protecting the plasma membrane of cells in landrace pig spermatozoa. Toelihere (1993) stated that the bottom of the abnormality is good ranging from 5-20%. So the highest abnormal percentage was in the P0 treatment, which was 7.80±2.38%, P3 6.80±0.83%, then P1 7.60±1.51% and P2 7.20±1.92% had lower values compared to the others. These results are consistent with the report of Hermawan *et al*. (2019) which said that actually the addition of EDKS in the HCS diluent to the abnormalities of landrace pig spermatozoa showed the best percentage value compared to the P0 treatment. This observed abnormal value provides information that the addition of EDKS has a positive effect to suppress the rate of increase in spermartozoa abnormalities during storage. This may happen because fresh moringa leaves contain antioxidants that are able to suppress free radicals. In general, the percentage of abnormality values of each treatment in masi research is suitable for use in artificial insemination, because the abnormality value of masi is in the normal range, which is less than 20% of abnormal spermatozoa morphology Susilawati (2011).

The cause of the decrease in abnormalities is due to the length of storage time which affects the number of abnormalities of spermatozoa in Solihati and Kuna (2009). The decrease in the abnormal presntase is suspected to be due to the anti-oxidant content contained in moringa leaves which affects the spermatogenesis process and reduces oxidative stress, resulting in sperm cells that are more resistant during the dilution process. The content of vitamin c functions as an antioxidant that can ward off free radicals so that the spermatozoa cell membrane remains protected and minimizes the level of abnormalities (Nugraheni *at al*., 2003). However, there are other factors that cause high abnormalities such as environmental factors, sample storage and preparation. Environmental factors such as temperature changes can cause tail abnormalities. The average form of abnormality during the study is the remaining abnormalities such as a circular tail, a broken tail, a folded tail. So in general, abnormalities in spermatozoa can be caused by various factors including genetics, stress, temperature, attachment and even treatment at the time of freezing semen (Arifiantini and Ferdian, 2006). Spermatozoa remain intact or do not increase high enough after dilution due to the content of lipoproteins and lecithin in egg yolks can stop the damage to the plasma membrane. In spermatozoa by maintaining and maintaining the integrity of the plasma membrane cavity (Manehat *et al*., 2021).

Spermatozoa Survival

Spermatozoa survival is the ability of spermatozoa to survive after post-dilution to be able to move for a certain period of time. The survival of spermatozoa in each treatment can be seen in this graph



At the above analysis stage, it was shown that the treatments P0 (14.40±3.57) were significantly different (P<0.05) from P1 (27.44±2.73), P2 (27.73±4.04), P3 (24.40±7.19) and P4 (23.63±7.06), but P1, P2, P3 and P4 were not different in definition (P>0.05) from the results of the observations above.

The results of this observation showed that lanrace pig spermatozoa diluted with moringa leaf extract, NACl and egg yolks had a longer shelf life when compared to the control percentage (without the addition of moringa, NACl). From the results of this

examination, it is proven that with the addition of fresh EDK with P2 treatment (2%) it is still able to maintain life expectancy until the 27th hour of observation. This situation is caused because moringa leaf extract contains enough antioxidants to be able to suppress and inhibit the decrease in survival rate in landrace pig spermatozoa, while lecithin and lipoprotein in egg yolks are able to act as buffers that can prevent cold *shock* caused by a sudden drop in temperature. The percentage decrease in P4 (23.63±7.06) was caused by a by-product in the form of lactic acid which occurred due to the result of fructose metabolism. High lactic acid can have a negative impact on metabolic processes due to lipid peroxidation so that spermatozoa cells are damaged and die continuously. Hine, (2014) stated that one of the causes of spermatozoa death is due to the absence of protective elements in the semen plasma so that when spermatozoa are stored at low temperatures, cell membrane damage occurs and results in death.

The lifespan of landrace pig spermatozoa in this study was shorter, because the lack of nutrients and energy substrates provided was not able to meet the needs perfectly. Fresh moringa leaves contain tannin compounds in addition to being antioxidants, these compounds can also damage the plasma membrane and sperm mitochondria. According to Susetyarini (2013) a process involving the oxidation of free radicals and the increase of the bacase radicals, tannin compounds have the potential to damage the structure of the plasma membrane of mitochondria spermatozoa. Pleated peroxide increases the production of free radicals which results in a decrease in spermatozoa motility. If the plasma membrane structure blade of the spermatozoan mitochondrial plasma is damaged, then the sperm will lose the source of energy to move. This causes the survival of spermatozoa to decrease faster.

CONCLUSION

Based on the results of the above research, it was concluded that the addition of fresh moringa leaf extract in purebred chicken egg yolk thinner in maintaining the quality of landrace pig semen. It was concluded that the addition of Fresh Moringa Leaf Extract to egg yolks and NACl could maintain the quality of landrace pig spermatozoa even at the highest level (40%).

SUGGESTION

With this study, it is recommended to conduct further research to test the success of the implementation of artificial insemination (IB) using doses of 2.5% and 4% fresh moringa leaf extract in purebred chicken yolk retailers.

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