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Addition of Tomato Juice as Additive in Diluent of Egg Yolk Citrate on the Quality of Pesisir Cattle Semen at 5°C Storage Temperature

Tinda Afriani^{1)*}, Jaswandi¹⁾, Adisti Rastosari¹⁾, Muhammad Cadillac Al Razzak³⁾, Dwiki Wahyudi²⁾

¹⁾ Animal Biotechnology, Faculty of Animal Science, Andalas University

²⁾ Animal Science, Faculty of Animal Science, Andalas University

³⁾ Animal Production Technology, Faculty of Animal Science, Andalas University

*E-mail: tindaafriani@ansci.unand.ac.id

ABSTRACT

This study aims to determine the effect of adding tomato juice as an additive in egg yolk citrate diluent on the quality of Pesisir cattle semen at 5°C storage temperature. The study was conducted experimentally in the laboratory consisting of 3 treatments, namely P0 (100% Egg Yolk Citrate), P1 (90% Egg Yolk Citrate + 10% Tomato Juice) and P2 (80% Egg Yolk Citrate + 20% Tomato Juice), then equilibrated at 5°C for 4 hours and each treatment was repeated 5 times. The observed variables included spermatozoa motility, spermatozoa survival, spermatozoa abnormalities and intact plasma membranes of spermatozoa. The obtained data was analyzed with the Analysis of Variance (ANOVA). The results showed that the addition of tomato juice extract into egg yolk citrate diluent on motility, percentage of survival and percentage of intact plasma membranes showed a very significant difference ($P < 0.01$), however, the abnormality had no significant effect ($P > 0.05$). The addition of 20% tomato juice extract in egg yolk citrate diluent showed better results with motility values is 72.00%; a percentage of life is 79.30%; intact plasma membrane is 80.39% and abnormality is 6.22%.

Kata kunci : *tomato juice, egg yolk citrate, Pesisir cattle, equilibration.*

INTRODUCTION

Pesisir cattle are native Indonesian cattle whose distribution is in the Province of West Sumatra. This cattle has advantages over other cattles, because the Pesisir cattle has a very high body resistance and environmental adaptation. However, every year there is a decline in the population of Pesisir cattle. This requires special attention in increasing the population of Pesisir cattle. One of them is the application of Artificial Insemination.

The processing and dilution of semen can reduce the quality of semen, due to contact with air so that lipid oxidation reactions occur which can cause damage to the plasma membrane. This damage is caused by the

formation of free radicals from spermatozoa metabolism, one of which is the level of Reactive Oxygen Species (ROS). If the initial ROS reaction is not controlled, a continuous reaction (autocatalytic) will occur (Suryohudoyo, 2000).

The high levels of ROS can be overcome by adding additives containing antioxidants to the semen diluent. High enough antioxidants can be found in tomatoes (*Solanum lycopersicum*). The antioxidant content in tomatoes is a carotenoid compound called lycopene. According to Suciati (2012), lycopene has a unique chemical structure, namely acyclic beta-carotene without provitamin A activity and is more efficient at scavenging free radicals than other carotenoids. In addition, Sumardiono et al. (2009) stated that the juice from tomatoes

contains various nutrients such as carbohydrates, protein, vitamin A, vitamin C and lycopene which function as antioxidants.

Carbohydrates and antioxidants found in tomato juice can serve as a provider of energy sources and free radicals that can damage cells. Rosmaidar et al. (2013) stated that the addition of 20% tomato juice in 100 ml of egg yolk citrate diluent carried out on Boer goat semen resulted in the best quality of spermatozoa after 72 hours of storage. This study describes the effect of adding tomato juice in egg yolk citrate with different doses on the semen quality of Pesisir cattle at 5°C storage including motility, live percentage, abnormalities and intact plasma membrane.

MATERIAL AND METHODS

This research was conducted experimentally at the Laboratory of Animal Biotechnology, Faculty of Animal Husbandry, Andalas University.

Material

The materials used were fresh semen from Pesisir cattle, a set of artificial vaginal apparatus, a collection tube, a thermometer, aluminum foil, tomatoes, digital scales, beaker glass, funnel, test tubes, filter cloth, filter paper, Erlenmeyer, 2.9 g Na-citrate, 100 ml aquabides and 4:1 egg yolk citrate, penstrep-400 antibiotic, pH meter, microscope, object glass, cover glass, pipette, microtube, 2% eosin, HOS solution, formolsalin and aquadest, using 5°C storage room.

Methods

This study used a randomized block design (RAK) with 3 treatments and 5 replications. Each treatment was analyzed using the analysis of variance (ANOVA) method. If the treatment showed significantly different results ($P < 0.05$), it was necessary to carry out further

tests using Duncan's Multiple Range Test (DMRT) according to Steel and Torrie (1995).

The process of making tomato juice: Prepared tools and materials for extracting tomato juice. Tomatoes were washed thoroughly, then cut into cubes. Put in a blender and then blended until smooth. After blending, the tomatoes were filtered using a filter cloth and inserted into a 250 ml erlenmeyer, the results of the filtering are allowed to form a supernatant in an Erlenmeyer, then take part of the supernatant in an erlenmeyer and then transferred to a sterile test tube.

The process of making egg yolk citrate diluent is by dissolving 2.9 grams of Na with 100 ml of aquabides. The mixed solution was cooled to room temperature. Furthermore, egg yolk is added in a ratio of 4: 1. The egg yolk citrate diluent is divided into 3 parts, then put into a microtube with 1 ml of diluent for each treatment:

- a. P0 : egg yolk citrate diluent without adding tomato juice.
- b. P1 : Added tomato juice extract with a concentration of 0.1 ml into 0.9 ml of egg yolk citrate diluent and then homogenized.
- c. P2 : Added tomato juice extract with a concentration of 0.2 ml into 0.8 ml of egg yolk citrate diluent and then homogenized.

RESULT AND DISCUSSION

Quality of Pesisir Cattle Fresh Semen

Testing the quality of fresh semen of Pesisir cattle includes macroscopic and microscopic examination. Macroscopic examination includes volume, pH, color, consistency and odor. While microscopically includes mass movement, motility, concentration, percentage of life, abnormalities of intact plasma membrane and intact acrosomal head. The quality of fresh semen of Pesisir cattle can be seen in table 1.

Table 1. Evaluation of the quality of Pesisir cattle fresh semen

Variable	The results of observations on the ejaculation				
	1	2	3	4	5
Volume (ml)	4	4	2	2	2
Color	Milky white	Milky white	Milky white	Cream	Cream
Consistency	Thick	Thick	Thick	Medium	Medium
pH	7	7	7	7	7
Mass Motility	+++	+++	+++	++	++
Motility (%)	80	80	80	70	70
Viability (%)	82,31	84,46	89,6	74,28	76,47
Abnormality (%)	5,80	5,70	5,06	6,92	5,70
Plasma Intact Membrane (%)	81,00	83,80	84,00	78,00	83,02
Concentration (milion/ml)	1140	1190	1365	850	940

The semen quality of Pesisir cattle was observed in ejaculation 1 to 5 (table 1) and the volume of semen of Pesisir cattle ranged from 2 ml to 4 ml. This result is lower than the semen of Brahman cattle which ranges up to 4.72 ml (Kuswahyuni, 2009) and other local cattle such as Bali cattle which has an average of 4.5 ml (Ratnawati et al. 2009). Low volume does not cause harm, but if the concentration is also low, it will cause a limited number of spermatozoa in one ejaculate (Feradis, 2010).

The color of the Pesisir cattle semen sample shows the dominant color is milky white and cream. According to Bearden et al. (2004), the color of cow semen that is normal is milky white and 10% is cream. These results are also similar to those reported by Muzakkir et al. (2017) who reported that Aceh cattle semen was a creamy white. The consistency of Pesisir cattle semen ranges from medium to thick. The thick consistency, the higher the concentration of spermatozoa (Feradis, 2010).

The observed consistency of Pesisir cows from 5 ejaculations had similar results to those reported by Muhammad et al. (2016), that the consistency of FH cattle semen ranges from thick to watery. The degree of acidity or pH is a factor that determines the life status of spermatozoa. If the pH is lower than normal, it will cause spermatozoa to die quickly. According to Toelihere (1985), the neutral pH in cattle and

sheep semen is around 6.2-7.5. The concentration of spermatozoa obtained ranged from 850×10^6 - 1365×10^6 /ml of semen with an average of 1097×10^6 /ml of semen, in accordance with Toelihere (1985) that the average concentration of spermatozoa cells in bovine semen ranged from 30 - 250×10^7 /ml of semen. Spermatozoa concentration calculations were carried out to determine the amount of diluent to be used.

Mass movement is indicated by the sign (+), by observing the mass waves observed using a microscope. The mass movement of Pesisir cattle samples in this study was ++ to +++ . This result makes it possible to carry out further processing because the cement requirements that can be processed have a mass movement of ++ (good) (Partodihardjo, 1992). The individual movement/motility of the Pesisir cow semen obtained in this study had an average of 76%. This result is the same as that reported by Dewi and Jumini (2012) who found the percentage of Bali cattle motility in Indonesia was 74.50%. This difference in results may be caused by differences in species, age, feed, maintenance management, frequency of collection and collection techniques (Hafez, 2000).

The percentage of live spermatozoa of Pesisir cattle in this study obtained an average

of 81.42%. These results are relatively the same according to Ratnawati et al. (2009) who reported that the average percentage of live spermatozoa in Bali cattle was 88.03% and according to Sukmawati (2014) who reported the percentage of live spermatozoa in limousine cattle, which was 94.08%. The percentage of live spermatozoa has a higher yield than the percentage of motility because motile spermatozoa are definitely alive, but live spermatozoa are not necessarily motile.

The abnormality of the semen of Pesisir cattle obtained in this study was an average of 5.84%. This result is relatively the same as the Bali cattle reported by Afrianiti (2012) that the percentage of abnormality is 6.56%. The integrity of the plasma membrane obtained in this study was 78%-84% with an average of 81.96%. This result is relatively lower than the intact plasma membrane of Bali cattle spermatozoa with an average of 92.67% (Ratnawati et al., 2009). The percentage of the intact plasma membrane is positively correlated

with the level of fertility, the higher the intact plasma membrane, the higher the fertility.

Quality of Semen After Equilibration

Semen quality of Pesisir cattle after equilibration at 5°C, semen that had been equilibrated was treated with the addition of tomato juice into egg yolk citrate diluent. Semen evaluation includes motility, survival percentage, abnormalities and intact plasma membrane. The results of the evaluation can be seen in Table 2.

Semen Motility After Equilibration

The percentage of semen motility after equilibration can be seen in Table 2. The highest average motility was found in P2 with the addition of tomato juice extract with a concentration of 0.2 ml into 0.8 ml of egg yolk citrate diluent of 72.00±4.47%. While the lowest percentage was P0 without the addition of tomato juice extract/100% citrate Egg yolk (control) with a motility percentage of 60.00±0.00.

Table 2. Quality of Pesisir cattle semen with addition of tomato juice in egg yolk citrate diluent after equilibration.

Variable	Treatments		
	P0	P1	P2
Motility (%)	60,00±0,00 ^A	70,00±0,00 ^B	72,00±4,47 ^C
Viability (%)	62,79±4,11 ^A	76,19±4,55 ^B	79,30±4,54 ^B
Abnormality (%)	6,04±1,34	6,98±1,48	6,22±0,93
Plasma Intact plasma (%)	65,60±3,71 ^A	78,03±2,33 ^B	80,39±2,29 ^B

Description : Superscripts with different capital letters on the same line showed a very significant difference (P<0.01)

Based on the results of ANOVA analysis, it was shown that the addition of tomato juice extract to the percentage of spermatozoa motility after equilibration showed a very significant difference (P<0.01). DMRT further test showed that the treatment was very significantly different between P0, P1 and P2 (P<0.01). The addition of 20% tomato juice extract (P2) was able to maintain better motility

than control (P0). These results are relatively similar to the research of Rosmaidar et al. (2013) who used different tomato juice 0%, 20%, 40%, and 80% in egg yolk citrate diluent as a boer goat semen diluent, the best result was the addition of 20% tomato juice in egg yolk citrate diluent. This is because the tomato juice extract contains an antioxidant in the form of lycopene which can reduce levels of ROS

(Reactive Oxygen Species) the effect of peroxide radicals so as to reduce the motility of spermatozoa stored in vitro. Antioxidants are very important to reduce the negative effects of ROS that can cause damage to spermatozoa cells (Pryor et al., 2000). According to Meo, (2022) described that lycopene can stabilize the molecular element oxygen (O) and prevent the effects of peroxide radicals so as to provide protection for cells and tissues against the negative effects of lipid peroxidation.

Viability of Semen After Equilibrasi

Based on Table 2, the percentage of live spermatozoa of Pesisir cattle with the addition of tomato juice extract in egg yolk citrate diluent was highest at P2 of $79.30 \pm 4.54\%$. While the lowest result is P0 of $62.79 \pm 4.11\%$. The results of the analysis showed that the results were significantly different ($P < 0.01$). Based on the results of the DMRT (Duncans Multiple Range Test) test, it showed that the treatment of P0 with P1 and P0 with P2 was very significantly different ($P < 0.01$). Meanwhile P1 and P2 treatments were not significantly different ($P \geq 0.05$). The low percentage of viable P0 is believed to be due to chemical damage caused by free radicals resulting from the metabolism of these cells during equilibration. Lipid peroxidation will occur due to a reaction between reactive oxygen species (reactive oxygen species) and unsaturated fatty acids that make up the plasma membrane of spermatozoa cells. The results obtained are also the same as those reported by Rosmaidar et al. (2013) that the best results were the addition of 20% tomato juice in egg yolk citrate diluent.

Semen Abnormalities After Equilibration

The results of observing the abnormality of Pesisir cattle semen after equilibration, obtained the highest result, namely P1 of $6.98 \pm 1.48\%$ and the lowest, namely P0 of $6.04 \pm 1.34\%$. These results are the same as those

of Meo et al. (2022) who reported spermatozoa abnormalities with the addition of tomato juice in egg yolk citrate diluent ranged from 7.06% to 7.28%. Based on the results of the analysis showed that the results were not significantly different ($P > 0.05$) on the spermatozoa abnormalities of Pesisir cattle. So it shows that the addition of tomato juice extract in egg yolk citrate diluent with different levels does not affect the percentage of abnormalities. Abnormalities are divided into 2, namely primary and secondary abnormalities. Solihati et al. (2008) stated that secondary abnormalities occur during passage through the epididymis, vas deferens and passage through the urethra and contamination with urine.

Intact Plasma Membrane Semen After Equilibration

The integrity of the plasma membrane of the equilibrated Pesisir cattle semen showed that the highest yield was P2 of $80.39 \pm 2.29\%$ and the lowest was P0 of $65.60 \pm 3.71\%$. This result is lower than that reported by Anwar (2004) who obtained the percentage of intact plasma membranes ranging from 88.88% to 92.74%. Based on the analysis, the addition of tomato juice extract in egg yolk citrate diluent to intact plasma membranes had a very significant effect ($P < 0.01$). The results of Duncan's test showed that the percentage of intact semen plasma membrane after equilibration had a very significant effect ($P < 0.01$) between P0 and P1 and P0 and P2, while P1 and P2 showed no significant effect ($P > 0.05$).

CONCLUSION

1. Based on research conducted on semen quality with the addition of tomato juice extract in egg yolk citrate diluent with 5°C storage the best is 20% tomato juice extract + 80% egg yolk citrate (P2) with a motility percentage of $72.00 \pm 4.47\%$,

the percentage of viability was 79.30 ± 4.54 and the plasma membrane intact was 80.39 ± 2.29 . Meanwhile, the best percentage of abnormality was in the treatment of 100% egg yolk citrate (P2) of 6.04 ± 1.34 .

2. Based on the results of this study, it can be concluded that the addition of tomato juice extract in egg yolk citrate diluent gave a very significantly different effect ($P < 0.01$) on the percentage of motility, viability and intact plasma membrane of Pesisir cattle spermatozoa, but the effect was not significant ($P > 0,05$) on spermatozoa abnormalities of Pesisir cattle.

7 ACKNOWLEDGEMENT

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