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# The Effect of Using Papaya and Watermelon Juice as a Non-Synthetic Buffer on the Quality of Kacang Goat's (*Capra Hircus*) Liquid Semen During Cold Storage at 5ºC

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**ABSTRACT:** This study examines the role of non-synthetic buffers from watermelon and papaya juice added to coconut water diluent on the liquid semen quality of Kacang goats during cold storage at 5oC for three months. An experimental method was used in which semen from a 2.5-year-old male was collected twice a week using an artificial vagina. The primary ingredients include young coconut water, Viridis, watermelon, papaya, NaHCO3, and Egg Yolk (KT). Furthermore, the observed variables were individual motility, viability, and abnormality. This study used a Randomized Block Design (RAK)) with 3 treatments and 10 replications, namely P0: Coconut Water + NaHCO3 + 10% KT, P1: Coconut Water + Watermelon Juice + 10% KT, and P2: Coconut Water + Papaya Juice + 10% KT. The data were analyzed using Variety Analysis. The results showed the treatment had a significant effect ($P<0.01$) on individual motility at all storage times, with the best motility up to 120 storage minutes with 61.00±3.16% at P1. Furthermore, the treatment had a significant effect ($P<0.01$) on viability at all storage times. The best treatment was P0 (72.24±5.28%), and the lowest was P2 (1.40±0.84%), with a shelf life of 120 minutes. The treatment had no effect ($P>0.01$) on abnormality at all storage times. It can be concluded that the use of watermelon and papaya juice as non-synthetic buffers in coconut water diluent on Kacang goat semen affects motility and viability but has no effect on abnormality.

**Keywords:** Buffer; Watermelon; Papaya; Semen; Kacang goat

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## INTRODUCTION

The efforts to increase the productivity and population of Kacang goats (Capra hircus) as germplasm in North Maluku through artificial insemination (AI) are constrained by the availability of frozen semen. It is because the manufacturing process requires equipment and the availability of liquid nitrogen, which is not easily imported into this area.

Therefore, an alternative approach is to use liquid semen processed from Kacang goats in North Maluku. Producing liquid semen from a technical point of view is easy and economically cheap because it uses coconut water as a primary diluent. Coconut water contains biochemical components that guarantee the physiological needs of spermatozoa, but it has a high acid content. There is an average pH between 5.2–5.5 (Campos et al., 1996; Barlina et al., 2007) in young coconut water, while old ones have an average of 6.0 (Barlina et al., 2007), which is biologically not suitable for the spermatozoa.

Therefore, it needs to be neutralized with buffering. The compound used to neutralize coconut water's acid content is $NaHCO_3$ (Sodium bicarbonate). This synthetic buffer material is at risk of exposure to spermatozoa with chemical residues, which decreases the physiological properties of the diluent used. Hence, alternative plant materials are used, namely watermelon and papaya extracted from the fruit juice.

Watermelon (*Citrulus lanatus*) has a water content of up to 92% of its weight, making it very alkaline (strong base) with low nutritional content. The vitamins and protein are low, while carbohydrates in sugar are only 7% (Tadmor et al., 2005). Another fruit that can be used is papaya (*Carica papaya* L) which has strong alkalinity. This study is a breakthrough in maximizing the use of coconut water as a primary diluent which is neutralized by using a non-synthetic buffer from watermelon and papaya juice, which makes it suitable as a buffer for spermatozoa. This study examines the role of non-synthetic buffers from the juice of Watermelon and Papaya fruit added to a coconut water base diluent on the liquid semen quality of Kacang goats during cold storage.

## MATERIALS AND METHODS

### Materials

The material was the fresh semen of a 3.5-year-old Kacang goat and one female angler. Semen collection was carried out twice a week. Furthermore, the coconut water used was young green coconut (Viridis), five watermelons and five papayas, egg yolk, 70% alcohol, eosin-negrosin, penicillin, streptomycin, NaHCO3, and 3% NaCl. The equipment used includes a light microscope, refrigerator, object-glass, cover glass, papaya, pH indicator, centrifuge, hemocytometer, Whatman filter paper, magnetic stirrer, empty straw, goat's artificial vagina, and hot plate.

### Methods

This study was conducted for three months with the following details, (1) pH analysis of coconut water, papaya, and watermelon in the laboratory of the Animal Husbandry Study Program, Faculty of Agriculture, Khairun University for one month, (2) quality analysis of goat's liquid semen in the Agrotechnology Laboratory, Faculty of Agriculture, Khairun University for two months.

This is an experimental study with two stages, including (1) a preliminary study on pH analysis of coconut water, papaya, and watermelon to determine the percentage addition of papaya and watermelon juice to 50 ml of coconut water, (2) analysis to determine the quality of goat's liquid semen after treatment. Furthermore, this study used a Randomized Block Design (RAK) (Yitnosumarto, 1993) with three treatments where each was repeated ten times, including P0: Coconut Water + NaHCO3 + 10% Egg Yolk, P1: Coconut Water + Watermelon Juice + 10% Egg Yolk, and P2:

Coconut Water + Papaya Juice + 10% Egg Yolk.

**Data Analysis**

The data were analyzed using Analysis of Variance (ANOVA) with 5% and 1% significance levels. When there was a difference between the treatments, Duncan's Multiple Distance test (Steel and Torrie, 1995) was performed using the Genstat Program 14.2. The variables observed in the quality test were individual motility, viability, and abnormality. The implementation of the study began with preliminary research, namely the determination of the percentage of adding non-synthetic buffers to 50ml coconut water diluent solution.

Furthermore, watermelon and papaya were squeezed and filtered with filter paper to obtain the juice. The pH was then measured with an indicator paper. The pH of the coconut water was also measured to determine the degree of acidity. It was subsequently neutralized to pH 6.8-7.00 by gradual addition until the concentration reached the ideal. The papaya and watermelon juice was inactivated at 56oC for 20 minutes.

This neutral and inactivated solution was used as a sample to be added with 10% egg yolk as a sample for the semen quality test. (1.) Control Treatment (P0): Coconut Water + NaHCO3 + 10% Egg Yolk. Coconut water was filtered with Whatman filter paper to remove impurities and placed in a 500 ml beaker glass labeled according to the treatment. It was inactivated by heating at a temperature of 56oC for 20 minutes, then 100 ml was taken for the sample-making process, starting with measuring acidity using a pH indicator.

Furthermore, 0.2 grams of NaHCO3 was given as a synthetic buffer until the pH of the coconut water became alkaline at 6.8 – 7.00. When used for sample preparation, 10% egg yolk was mixed as an extracellular cryoprotectant. It was stirred with a magnetic stirrer for 15 minutes until the solution became homogeneous and centrifuged two times each for 30 minutes at a speed of 1500 rpm. The supernatant was placed in new tubes.

Subsequently, 5 ml was respectively poured into the two new test tubes as a sample which would later be mixed with fresh semen. (2) Treatment P1: Coconut Water + Watermelon Juice + 10% Egg Yolk. Coconut water was filtered with Whatman filter paper to remove impurities and placed in a 500 ml glass beaker labeled according to treatment. It was inactivated by heating at a temperature of 56oC for 20 minutes, then 100 ml was taken for the sample-making process, starting with measuring the acidity using a pH meter.

Furthermore, a non-synthetic buffer from watermelon juice was given until the pH of the coconut water became alkaline which was marked by a pH of 6.8 – 7.00. When used for sample preparation, 10% egg yolk was mixed as an extracellular cryoprotectant. It was stirred with a magnetic stirrer for 15 minutes until it became homogeneous and centrifuged two times each for 30 minutes at a speed of 1500 rpm.

The supernatant was placed in new tubes. Moreover, 5 ml was respectively poured into the two new test tubes as a sample which would later be mixed with fresh semen. (3) Treatment P2: Coconut Water + Papaya Juice + 10% Egg Yolk. The working procedure in P2 was the same as in P1, only replacing watermelon juice with papaya juice. Subsequently, all samples were analyzed every 30 minutes for up to 2 hours of cold storage.

## RESULT AND DISCUSSION

**Semen Examination**

The semen examination of the Kacang goat was carried out using an artificial vagina (VB) initiated by performing false mounting three times using a 3-year-old female goat teaser. The semen examination results before treatment collected two times a week have the mean, as shown in Table 1.

**Table 1.** Data of Mean Fresh Semen in Kacang Goat

| Parameter | Mean (±) SD |
|---|---|
| Volume (ml) | 0.66 ± 0.1 |
| Color | Beige |
| pH | 7 |
| Consistency | Thick |
| Smell | Typical semen |
| Mass motility | 3+ |
| Individual motility (%) | 79 ± 2 |
| Concentration (million/ml) | 397.0 ± 10.94 |
| Viability (%) | 88±1.62 |
| Abnormality (%) | 1.6 ± 0.25 |

After storage before preparation for the sample, the average volume of semen at 0.66 ml ± 0.1 is obtained. This result is lower than that of Pamungkas et al. (2009) at 0.77 ml ± 0.28 in the fresh semen of the Kacang goat. This result is also lower than Setiawan and Kusumawati (2017) in Kacang goat semen in Blitar, East Java, where the volume is 1 ml. It is slightly higher than Bintara (2011) in Yogyakarta, with a volume of 0.62 ml±0.2.

Meanwhile, the color, pH, consistency, smell, and mass motility are almost identical to Setiawan and Kususmawati (2017). Individual motility, viability, and abnormality are lower than Setiawan and Kusumawati (2017) by (94.8%), (96.4%), and (2.9%). Furthermore, the concentration is higher than Setiawan and Kususmawati (2017) by (3540.2x106/ml). The differences in volume, individual motility, viability, abnormality, and concentration are caused by variations in the goats, body weight, libido, quantity, age of males, and quality of feed. The local Kacang goat used in this study has a smaller body weight (20kg average) with a small body and testicular volume.

**Individual Motility**

Observation of spermatozoa motility in the liquid semen was carried out using a phase-contrast microscope with 400 times magnification.

**Table 2.** Average sperm motility (%)

| Treatment | Observation time (minutes) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | 120 |
| P0 | 69.50±1.58c | 68.50±3.37c | 63.50±4.12c | 61.00±3.16c |
| P1 | 50.00±0.00b | 37.00±3.50b | 24.00±4.59b | 6.00±2.11b |
| P2 | 40.00±0.00a | 23.00±2.58a | 5.00±0.00a | 0.00a |

Note: Different notations in the same column indicate a significant difference between treatments (P<0.01).

The analysis of variance (ANOVA) showed that the treatment has a very significant effect (P<0.01) on the spermatozoa's individual motility at all times during cold storage. Furthermore, the treatment of coconut water + NaHCO3 + 10% egg yolk has the highest motility percentage up to a storage time of 120 minutes which reaches 61.00±3.16% compared to the motility in the treatment of coconut water + watermelon juice + 10% egg yolk (6.00±2.11%) and coconut water + papaya juice + 10% egg yolk (0.00%). This result is slightly lower than Salim et al. (2019), which reached 71.5 ± 4.1 for a storage time of 1 day in coconut water + 10% egg yolk treatment with the addition of NaHCO3 as a buffer. Meanwhile, between P1 and P2, the best average percentage is 50.00±0.00% in P1 and 40.00±0.00% in P2

at 30 storage minutes. This happens because the pH of watermelon juice is six while papaya juice is 5. This condition affects the movement activities of spermatozoa. The low individual motility in P1 and P2 is due to the synthetic buffer from watermelon and papaya juice which cannot neutralize the acidity of coconut water. Based on the preliminary results, the average pH of coconut water is five, while watermelon and papaya are 6 and 5, respectively. Due to the non-acting of the coconut water non-synthetic buffers that changed the solution from acidic to alkaline during storage, there was a drastic decrease in the percentage of spermatozoa motility during storage for 2 hours. The spermatozoa cannot survive and carry out movement activities when the diluent medium is not alkaline. According to Holm and Wishart (1998), the percentage and speed of motile sperm increase at an alkaline pH. Furthermore, Mishra et al. (2018) stated that motility decreases when pH is low. With decreasing pH from 6.5 to 6, the motility decreases linearly, indicating a potential role of pH in regulating sperm motility.

Table 3. Average sperm viability (%)

| Treatment | Observation time (minutes) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | 120 |
| P0 | 82,00±2,49b | 78,32±3,80c | 74,10±4,07c | 72,24±5,28c |
| P1 | 60,04±0,49a | 46,52±3,98b | 34,09±5,01b | 12,44±4,15b |
| P2 | 55,70±6,29a | 31,70±1,70a | 11,70±1,89a | 1,40±0,84a |

Note: Different notations in the same column indicate a significant difference between treatments (P<0.01).

The ANOVA results showed that the treatment has a very significant effect (P<0.01) on spermatozoa viability at all storage times. The best treatment is P0 with the highest viability percentage of 72.24±5.28% while the lowest is P2 by 1.40±0.84% at 120 storage minutes. This result is slightly better than Azis et al. (2018), who used red and green coconut water for two storage hours with red coconut (rubescent) at 70.24±13.89% and green (Viridis) at 70.70±17.94% were both used NaHCO3 buffer.

It is also higher than Audi et al. (2017), who used NaHCO3 as a buffer for water-based coconut diluent for Boer goat semen, where the viability of up to 2 storage hours was 58.59 ± 4.59 for old Viridis coconut water and 66.14 ± 12.27 for the young one. For the watermelon (P1) and papaya (P2) juice treatments, the highest average viability percentage is P1 of 82.00±2.49, and the lowest is P2 of 55.70±6.29 at 30 storage minutes. Statistically, P1 and P2 have no difference in storage time of 30 minutes. The low percentage of live spermatozoa in P1 and P2 is due to coconut water not buffering even though it has been added with watermelon and papaya juice. This is because the juice has a pH that is not much different from the primary diluent. This causes spermatozoa to be unable to maintain their physiological functions. The acidic environment of the diluent can cause damage to the sperm membrane. This is at risk of interfering with spermatozoa's intracellular and extracellular metabolism, hence the longer lifespan. According to Susilawati (2017), a suitable diluent should be buffering. The purpose of adding a non-synthetic buffer to the treatment is to avoid neutralizing the acidic properties of the primary diluent, thereby affecting the physiological functions of the spermatozoa. This will result in low viability of the treated sperm.

**Table 4.** Average sperm abnormality (%)

| Treatment | Observation time (minutes) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | 120 |
| P0 | 1.47±0.62 | 1.66±0.61 | 2.04±0.55 | 2.82±1.09 |
| P1 | 1.26±0.54 | 1.72±0.60 | 2.03±0.65 | 2.78±0.80 |
| P2 | 1.40±0.61 | 1.50±0.64 | 1.92±0.74 | 3.84±1.26 |

Based on the analysis of variance results, the treatment has no effect on spermatozoa abnormality at all storage times. Furthermore, the treatments have no difference (P>0.05). This result follows Mugiyati et al. (2017), who reported no difference between treatments during storage for five days using coconut water diluent buffered with NaHCO3 in the liquid semen of Boer goat.

This condition showed that using non-synthetic buffers from watermelon and papaya juice and synthetic buffers of NaHCO3 has no effect on spermatozoa abnormality from 30 minutes to 2 hours of storage. However, there is an increase in the abnormality with increasing storage time. This is due to the acidic conditions of the diluent because the watermelon and papaya juice prevented it to be buffered, thereby causing damage to the tail and head released. However, this abnormality value is still far below the standard of 20%, which is not suitable for artificial insemination (AI). Susilawati (2013) stated that when the semen abnormality is 15%, it is unsuitable for AI.

## CONCLUSIONS

The use of watermelon (*Citrullus lanatus*. L) and papaya (*Carica papaya*. L) juice as a non-synthetic buffer in coconut water diluent for Kacang goat liquid semen during cold storage affects motility and viability of spermatozoa but does not affect abnormality.

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