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Evaluation of Green Coconut Water as an Additional Diluent on the Quality and Storability of Madenan Chicken Sperm

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ABSTRACT

Metode penelitian yang digunakan adalah eksperimen laboratorium dengan rancangan acak kelompok. Lama penyimpanan pada suhu dingin 5°C yaitu selama 0, 1, 2, 3, 4 jam. Perlakuan penelitian dengan mengencerkan semen pada media fisiologis NaCl yang dicampur dengan air kelapa hijau. Terdapat empat perlakuan yaitu L0 = NaCl fisiologis 100% + air kelapa hijau 0%, L1 = NaCl fisiologis 95% + air kelapa hijau 5%, L2 = NaCl fisiologis 85% + air kelapa hijau 15%, dan L3 75% fisiologis NaCl + air kelapa hijau 25%,. Variabel yang diamati adalah motilitas, viabilitas dan kelainan spermatozoa yang diperoleh dianalisis secara deskriptif. Data perlakuan dianalisis menggunakan ANOVA dan apabila terdapat perbedaan antar perlakuan maka dilakukan uji Duncan. Hasil penelitian menunjukkan bahwa kualitas dan kuantitas semen Ayam Madenan berpengaruh terhadap penambahan pengencer kelapa hijau. Hasil uji kualitas dan kuantitas semen. motilitas individu 86.20 ± 1.02 %, viabilitas 82,60±7,04, dan kelainan 7,50±2,20 %. Perlakuan memberikan pengaruh yang sangat nyata (P<0,01) terhadap motilitas spermatozoa. Kesimpulan dari penelitian ini adalah penggunaan air kelapa hijau sebagai pengencer semen sebanyak 5% perlakuan L1 lebih baik diantara perlakuan lain dapat menjaga kualitas spermatozoa Ayam Madenan. Motilitas yang diperoleh sebesar 55,50 ± 21,68 % dengan lama penyimpanan 4 jam pada suhu dingin 5^0C.

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ABSTRACT

The research method used was a laboratory experiment with a randomized block design, storage time at a cold temperature of 5°C, namely 0, 1, 2, 3, 4 hours. The treatment was by diluting the cement in the physiological medium NaCl mixed with green coconut water. There were four treatments, namely L0 = 100% physiological NaCl + 0% green coconut water, L1 = 95% physiological NaCl + 5% green coconut water, L2 = 85% physiological NaCl + 15% green coconut water, and L3 75% physiological NaCl + green coconut water 25%,. The observed variables are. The motility, viability and abnormalities of the spermatozoa obtained were analyzed descriptively. Treatment data were analyzed using ANOVA and if there were differences between treatments, the Duncan test was carried out. The results showed that the quality and quantity of Madenan chicken semen influenced the addition of green coconut thinner. Cement quality and quantity test results individual motility 82.60 ± 7.04%, viability 86.20 ± 1.02 %, and abnormalities 7.50 ± 2.20 %. The treatment had a very significant effect (P<0.01) on spermatozoa motility. The conclusion of this research is that the use of green coconut water as a semen diluent of 5% treatment L1 was better the other treatments can maintain the quality of spermatozoa in Madenan chickens. The motility obtained was 55.50 ± 21.68% with a storage time of 4 hours at a cold temperature of 5°C.

INTRODUCTION

People enjoy Madenan chickens because they are simple to raise. But as it developed, several flaws in madenan chicken were discovered, which could prevent it from being more productive. Zabig *et al*. (2017) lists these drawbacks as sluggish development and sexual maturity, as well as comparatively high prices brought on by strong demand that is not offset by the availability of Madenan Chicken. To boost animal productivity, biotechnology for reproductive purposes must be applied Artificial insemination (AI) is a type of livestock reproductive biotechnology that is often used to increase chicken productivity. The AI process includes collecting and diluting semen.

The semen dilution process requires a diluent that can maintain the quality and quantity of spermatozoa. Several studies have examined the role of vitamins and fructose as additional ingredients in maintaining the quality of spermatozoa during storage (Khaeruddin & Srimaharani, 2019). One of the semen dilution ingredients is green coconut water. The potential of coconut water has been widely studied as a source of vitamins and energy sources which has a fairly high vitamin C content of 22-34 mg/100 (Moghbeli *et al.*, 2016) and is rich in natural minerals (potassium, sodium chloride, magnesium, ferum cumprum, phosphorus, and sulfur), sugar (glucose, sucrose, fructose, sorbitol and inositol), nitrogen elements in the form of protein composed of amino acids (aline, arginine, cysteine, and serine) and vitamin C to help overcome the threat of environmental shock because it is isotonic. Based on the description above, it is necessary to research the use of coconut water as an alternative thinner for Madenan Chicken semen. The purpose of the research was to test and assess the effect of giving amounts of green coconut water as an extra ingredient in NaCl diluent on the quality and shelf life of Madenan chicken semen at cold temperatures.

METHODS

Two male Madenan roosters, around 9–12 months old, with a body weight of 2.5–3, kept in separate cages, were used as research subjects. They were in good reproductive health and had a strong libido. The semen storage process is carried out at 06.00 am. semen collection was carried out twice a week using the massage method. The cloaca was cleaned with tissue before semen collection to ensure that the semen was free of contamination. The freshly obtained semen was examined under a microscope to determine its volume, pH, odor, color, and consistency. Microscopic observations were carried out to calculate concentration mass motility, individual motility, viability and abnormalities.

The semen diluter was prepared from fresh cement with a ratio of fresh cement and diluent solution of 0.5:5. (cement 0,5 ml and diluent green cocounat water 5ml) Semen quality measurements were carried out every hour of storage. Individual spermatozoa abnormalities, viability, and motility were all evaluated under a microscope (Touazi, *et al*., 2018).

Volume, pH, color, consistency, odor, and mass movement are all included in the macroscopic assessment of semen. The semen volume can be directly seen on the scale tube. The pH of cement was measured with pH litmus paper. The color of the semen is observed directly on the spermatozoa or by looking at the color of the semen that has been collected (Zabig *et al*., 2017). The consistency or viscosity of the semen was tested by tilting the tube towards the spermatozoa. Once the tube was straightened again, if the semen in it dropped slowly, this suggested that the semen was thick in consistency.

The smell of cement was determined by smelling the surface of the tube (Mussa *et al*., 2021). The movement of the cement mass was rated as very good (+++) if there were large to small waves, thick and dark in large quantities and moving quickly; good (++) if the small waves were thin, rare and move forward slowly, moderate (+) if no visible waves but only individual progressive active spermatozoa movement, poor (O) or necrospermia (N) if little or no individual movement (Castillo *et al*., 2021).

The cement was dropped on a glass object and then evaluated under a microscope with a magnification of 10 x 40. Spermatozoa motility was checked from left to right to obtain 200 spermatozoa, then note their movement. The P value represents a progressive movement (moving forward quickly), C denotes circular movements (circling only in place), N is necrospermia, meaning no movement, and R is reverse movements (namely backward movement). Spermatozoa viability was determined by using an eosin nigrosine solution with a drop of spermatozoa. The eosin nigrosine solution and a drop of spermatozoa then was homogenized before it was smeared on an object glass, afterward it was observed under a microscope. Live spermatozoa appear white or clear while dead spermatozoa appear red or dark (Bonnefont *et al*., 2019). Observation of spermatozoa abnormalities was carried out by observing the morphology of the spermatozoa.

Data on spermatozoa motility, viability, and abnormalities obtained were analyzed descriptively. Treatment data were analyzed using ANOVA and if there were differences for each treatment, Duncan's test was carried out according to the instructions of Boveda *et al.* (2020).

RESULT AND DISCUSIONS

The evaluation results of fresh semen from two male Madenan chickens from 5 shelters showed that the fresh semen met the requirements and was suitable for dilution. The evaluation results can be seen in the Table 1. The spermatozoa in one ejaculation, it will reduce fertility.

Table 1. Results of Macroscopic and Microscopic Evaluation of Fresh Semen from Madenan Chickens Before Diluting

Parameter	Average
Volume (ml)	0.75 ± 0.08
Individual Motility (%)	80,70±4,71
Consistency	Medium
Mass Motility	(++/+++)
Sperm Viability (%)	82,60±7,04
Sperm Concentration (10^6 /mil)	243x107/ml
pH	8.3 ± 0.27
Abnormality (%)	7,50±2,20

Resource: Laboratory Production Animal IPB (2023)

Motility assessment involves subjective estimation of spermatozoa viability and motility quality. The average percentage of spermatozoa motility of Madenan chickens in the three groups after treatment can be seen in Table 2.

Tabel 2. Effect of Storage Time and Dosage of Green Coconut Water on The Progressive Motility of Individual Spermatozoa in Liquid Semen in Male Madenan Chicken

Treatment Dose	Storage Time (Hour)				
	0	1	2	3	4
L^0	71.00±16,73^a	70.00±20,16^a	65.00±18,62^{ab}	60.50±19,80^b	50.50±24,65^b
L^1	73.00±16,81^a	71.00±1834^a	70.00±17,94^b	61.00±20,59^b	55.50±21,68^{ab}
L^2	72.50±16,68^a	70.00±17,94^{ab}	66.00±18,34^b	60.50±19,80^b	52.50±25,06^b
L^3	70.50±19,24^a	69.50±16,34^{ab}	65.00±17,85^b	60.50±19,80^b	51.00±25,10^b

Note: Different notations in the same column show very significant differences (P≤0.01)

The average volume (0.75 ± 0.08 ml) and semen pH (8.3 ± 0.27) obtained in this study were in the normal category. The volume of poultry semen produced in one ejaculation is 0.2-0.5 ml or 0.3-1.0 ml per reservoir. According to Das (2021), the pH of the semen of Madenan Chickens varies between 8.5-9.0, while Asmarawati *et al.* (2019) stated 77.57 ± 3.67%. This good motility allows spermatozoa cells to reach the egg cells in that the average pH of poultry semen is 7.0-7.6. The results of spermatozoa motility were the oviduct within a relatively normal time, thus allowing complete fertilization to occur. Ibarra *et al.* (2020) stated

that normal poultry semen has individual motility between 60-80%. The average percentage of viable spermatozoa in the semen samples studied was 83.87 ± 2.22%. Normal cement has a viability of around 60-80% (Zabig *et al*., 2017). Normal semen means semen which, after microscopic evaluation is carried out based normal, by the opinion of Adeoye *et al*. (2017) which states that in most ejaculates the percentage of abnormal spermatozoa ranges from 5-20%, if abnormal spermatozoa are more than 20% of the total on differences in the affinity for, absorbing eosin-negrosine substances by spermatozoa, has a minimum survival percentage of 50%.

The abnormality of Madenan chicken spermatozoa obtained was 6.80 ± 0.78% (Table 1). This percentage is classified as Throughout four hours of observation, the average motility of Madenan chicken spermatozoa in all treatment groups generally decreased. The lengthy storage period in Madenan Chicken contributed to a decline in spermatozoa motility, which in turn affected the proportion of spermatozoa motility. The findings of the observations at 0, 1, 2, 3, and 4 hours indicated that L^1 had the most motility, whereas L^0 had the lowest. The study of spermatozoa motility data revealed an interaction between diluent material and storage time, as well as a highly significant difference ($p<0.01$) between motility and storage time. This shows that the effect of diluents on spermatozoa motility is influenced by the length of storage time. According to Mussa *et al*. (2021) due to the decline in the quality of spermatozoa, the energy supply also decreases. During storage, spermatozoa continue to carry out activities such as movement and metabolism. Because spermatozoa continue to undergo both aerobic and anaerobic metabolism while being stored, the pH level reduction is also greater. Haryuni *et al*. (2020) stated that spermatozoa metabolism in anaerobic conditions produces lactic acid which accumulates and reduces the pH of semen which ultimately reduces the motility and viability of spermatozoa. According to Asmarawati *et al*. (2019), different environmental changes will affect the quality of spermatozoa. Fresh semen from the liquid environmental conditions resulting from the secretion of the male genital glands to the diluent fluid used as well as the conditions for the balance of spermatozoa cells during the dilution process. This situation can result in shock to the spermatozoa cells so that individual motility decreases.

Miranda *et al*. (2018) stated that temperature changes will affect spermatozoa metabolism which results in energy production, which can be used as mechanical energy (movement) or as chemical energy (biosynthesis). The effect of diluents on spermatozoa motility differed between observation times. At 0 to 4 hours of observation, spermatozoa motility in treatment L1 was higher compared to L0 and L3. This is possible because L1 contains physiological NaCl, a solution that has pH buffering capacity (Blank *et al*., 2021) and is isotonic so that it can support spermatozoa motility for a longer time. The results of spermatozoa motility in Madenan chickens diluted with 95% physiological NaCl with the addition of 5% green coconut water

showed that spermatozoa motility was greater than all treatments for 4 hours with an average motility of 55.50 ± 21.68%. This result is not much different from the research results of Mussa *et al.* (2020) on the motility of Manila duck spermatozoa diluted with physiological NaCl still reached 44 ± 3.79% for 4 hours.

The motility of spermatozoa diluted 95% with the addition of 5% green coconut water (L0) was able to last for 4 hours with a value of (50.50 ± 24.65%). Likewise with L1, the value lasted for 4 hours (52.50 ± 25.06%). This is assumed to be the case because the coconut water in L2 and L3 has a relatively high mineral content, which causes the pH of the water to tend to be acidic (Silyukova *et al*., 2022). pH is one of the benchmark factors for influencing the viability of spermatozoa so it influences spermatozoa metabolism.

Viability of Liquid Semen

The results of observations of the average percentage survival rate of spermatozoa for Madenan chickens from each treatment during the study can be seen in Table 3.

Tabel 3. Effect of Storage Time and Dosage of Green Coconut Water on Viability Liquid Semen Spermatozoa in Male Madenan Chickens

Treatment Dose	Time Storage (Hour)				
	0	1	2	3	4
L0	90.12±0,33	89.62±0,36	88.98±0,10	88.68±0,40	88.59±0,19
L1	90.33±0,76	89.96±1,03	89.15±0,35	88.93±0,17	88.75±0,39
L2	90.28±0,57	89.95±0,97	89.06±0,20	88.84±0,40	88.66±0,39
L3	90.21±0,36	89.66±0,60	89.05±0,31	88.71±0,41	88.62±0,56

The results of the analysis of variance showed that the treatment had a very significant influence ($P<0.01$) on the survival rate of spermatozoa. After carrying out an analysis of variance, it showed that the survival rate of spermatozoa in the green coconut water treatment from (L0) to (L3) was statistically very significantly different. This is because the green coconut water solution has a nutritional substrate for spermatozoa, namely glucose, and an energy source. Glucose is one of the sources of compounds found in seminal plasma which functions as an energy source for spermatozoa. Furthermore, Santos *et al*. (2020) stated that the addition of glucose in the diluent is very useful and helps the survival of spermatozoa. The data in Table 3 shows that spermatozoa are still suitable for artificial insemination up to four hours of storage with the L0 - L3 treatment can be used until the 4th hour. The L2 and L3 diluent treatments mean the percentage of live sperm presented in Table 3 shows that the diluent treatment L1, L2 and L3 can maintain a higher percentage of live sperm compared to L0. This is thought to be due to the provision of different nutritional content in each diluent. Live spermatozoa in 100% coconut water (L0) diluent had the lowest percentage, this was because coconut water was unable to protect sperm from the effects of cold shock. Reduced energy in the diluent, decreased pH, toxic effects on seminal plasma, and osmotic pressure from the diluent can reduce semen quality (Behnamifar *et al*., 2021). Data in Table 3 shows that longer storage times reduce the number of live sperm in all treatments. The

results of research by Esguerra *et al*. (2020) show that the length of semen storage affects the percentage of live sperm. This statement is strengthened by Puja *et al*. (2018) that the percentage of live spermatozoa in semen diluted with NaCl and coconut water begins to decrease from the beginning of 0 hours slowly until the fourth hour of storage. The percentage of live spermatozoa which is still high at the start of storage is due to the availability of the required energy substances, the environmental conditions of the solution which are still stable, the osmotic pressure which is still isotonic, and the age of the spermatozoa which are still fresh.

Liquid Semen Abnormality

The average abnormalities in goat spermatozoa from each treatment during the study can be presented in Table 4.

Tabel 4. Effect of Storage Time and Dosage of Green Coconut Water on Abnormality Liquid

	Time Storage (Hour)				
Treatmen Dose	0	1	2	3	4
L0	0.98±0,48^{a}	1.82±0,41^{ab}	1.95±0,48^{ab}	2.95±1,18^{ab}	3.13±1,08^{b}
L1	0.86±0,39^{a}	1.64±0,52^{b}	1.73±0,25^{ab}	2.57±1,04^{b}	3.04±1,05^{b}
L2	0.95±0,47^{a}	1.75±0,56^{b}	1.81±0,42^{ab}	2.81±1,24^{b}	3.06±1,04^{b}
L3	0.97±1,41^{a}	1.79±0,39^{b}	1.83±0,38^{b}	2.87±1,46^{b}	3.11±1,07^{b}

Note: Different notations in the same column indicate differences very significant (P≤0.01)

The results of the analysis of variance showed that the treatments had a very significantly different effect (P<0.01) on spermatozoa abnormalities. This is because the physiological NaCl diluent is 0.9%, green coconut water is composed of ingredients that have a composition that is relatively isotonic with body fluids and seminal plasma. Green coconut water solution makes it possible to maintain abnormal spermatozoa. However, lactic acid is needed to meet the need for sodium bicarbonate ions which function to maintain the acidity of the solution or as a buffer solution and can maintain the osmotic pressure of the solution (Zadeh *et al*., 2020).

The results of diversity analysis showed that spermatozoa abnormalities in treatments (L0) and (L3) were very significantly different and lower than in treatment (L1). The use of green coconut water diluent gave the best results because it did not cause much morphological damage to spermatozoa with the lowest percentage of abnormalities. This is because coconut water contains glucose which is needed for spermatozoa metabolism and is thought to be able to maintain their life, especially for spermatozoa that are stored at cold temperatures. The high level of abnormality is caused because the level of fructose in the green coconut water diluent is too high, containing lactic acid which can lower the pH and can also be toxic to spermatozoa. So it causes more morphological damage to spermatozoa. However, this percentage can still be used for artificial insemination because it is still far below normal standards. Spermatozoa abnormalities of no more than 15% can still be used for artificial insemination (Bekele *et al*., 2023). The abnormalities in question include coiled tails, broken tails and heads without tails.

CONCLUSIONS

The use of green coconut water as a diluent in semen as much as 5% can maintain the quality of madenan chicken spermatozoa. The motility obtained was 55.50 ± 21.68 % with a storage time of 4 hours at cold temperature.

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CONTRIBUTION STATEMENT

In this article, Nolasco da Costa acts as the main contributor and correspondence contributor, while Acacio Cardoso Amaral acts as a member contributor.

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