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Innovation of Sugar Cane Juice Extract with the Addition of Citrate-Yolk as a Natural Diluent on the Effect of Motility and Viability of Duck (*Cairina moschata*) Spermatozoa at UPTD Livestock Services of East Java Province

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Abstract | The problem of low semen quality in local poultry, including duck (Cairina moschata), is still a major obstacle in the application of artificial insemination (AI) technology. One potential solution is the use of natural diluents that are cheap, easy to obtain, and still effective. This study aimed to evaluate the effect of a combination of sugarcane juice extract and egg yolk-citrate as a natural diluent on the motility and viability of duck spermatozoa. The research method used a completely randomized design (CRD) with four treatments and four replicates, namely P0 (control/NaCl 0.9%), P1 (1:1), P2 (1:2), and P3 (1:3), with a comparison of 0.5ml of spermatozoa. Parameters observed were motility and viability of spermatozoa analyzed using ANOVA and Duncan's further test. P2 treatment produced the highest motility (61.96%) and the best viability (80.01%), with statistically significant differences compared to other treatments. The combination of sugarcane juice and citrate-egg yolk at a ratio of 1:2 proved effective in improving the quality of duck spermatozoa and is recommended as a natural diluent in local poultry artificial insemination programs.

Keywords | Duck, Natural Diluent, Sugarcane juice, Egg Yolk, Spermatozoa

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INTRODUCTION

Duck (*Cairina moschata*) is one of the local poultry that has great potential as a meat and egg producer, as well as being used in germplasm preservation through artificial insemination (AI) programs (Aerawata *et al.*, 2021; Restiadi *et al.*, 2020). However, duck productivity is still low, mainly due to limitations in reproductive efficiency (Ramahtia *et al.*, 2024; Rosyada *et al.*, 2023). One of the main obstacles in the implementation of Artificial Insemniation is the low quality of semen due to the unavailability of effective, cheap, and easily obtainable diluents by local farmers (Ducha *et al.*, 2013). The use of natural ingredients as semen diluents is increasingly in

demand because they are considered more economical, environmentally friendly, and have minimal side effects (Apriliana *et al.*, 2021). One potential natural ingredient is sugarcane juice, which contains glucose and fructose as an energy source to support spermatozoa motility (Rohmah et. al., 2021; Yonarta and Faqih, 2021). The combination of sugarcane juice with egg yolk which is rich in lipoproteins and phospholipids is believed to be able to protect cell membranes, spermatozoa and maintain viability during storage (Miguel-Jimenez *et al.*, 2020; Permana et. al., 2025) Previous research by Tanii *et al.* (2022) proved that the use of sugarcane juice in citrate-yolk diluent can increase viability and reduce the abnormality of Bali cattle spermatozoa (Januskauskas *et al.*, 1999).

Based on this, this study was conducted to evaluate the effectiveness of the combination of sugarcane juice and citrate-egg yolk as a natural diluent on the quality of duck spermatozoa, especially motility and viability parameters (Ribas-Maynou *et al.*, 2023). We aimed to provide an alternative diluent that is more applicable to farmers in supporting the success of Artificial Insemination in local poultry (Til *et al.*, 2016; Irvanto *et al.*, 2021).

MATERIALS AND METHODS

This study was carried out from May to June at the UPTD of the Livestock Service Office of East Java Province using healthy adult male duck semen. The study was designed using a completely randomized design (CRD) with four treatments and four replicates, namely P0 (NaCl 0.9% as control), P1 (citrate-yolk diluent + sugarcane juice 5:5), P2 (citrate-yolk diluent + sugarcane juice 5:10), and P3 (citrate-yolk diluent + sugarcane juice 5:15) with 0.5ml of spermatozoa as the comparator. Semen was collected using the female duck lure mating technique, then mixed with the diluent according to the treatment. Evaluation was carried out on two parameters, namely motility and viability of spermatozoa. Motility was observed as the percentage of non-progressive motile and immotile sperm, while viability was determined by spermatozoa moving forward progressively (straight). Observation data were analyzed using analysis of variance (ANOVA), and if there were significant differences between treatments ($p<0.05$), then continued with the Duncan Multiple Range Test (DMRT) to determine which treatments were significantly different.

RESULTS AND DISCUSSION

The results showed that the diluent treatment of a combination of sugarcane juice and citrate-egg yellow had an effect on the quality of duck spermatozoa, which was measured through two main parameters, namely motility and viability. Measurements were made on four treatments (P0, P1, P2, P3) with four replicates on each treatment. The highest sperm motility value was obtained in the P2 treatment (1:2 combination) at 61.96%, followed by P1 at 57.86%, P3 at 57.73%, and P0 (control) at 48.01%. Meanwhile, the highest sperm viability was also found in P2 at 80.01%, followed by P1 at 76.66%, P3 at 75.86%, and P0 at 66.89%. Based on the ANOVA test, it is known that the treatment gives a significant effect on both parameters ($p<0.05$), and the results of the DMRT further test show that P2 is significantly different from the other treatments. The following table presents the average observation results of motility and viability of duck spermatozoa in each treatment.

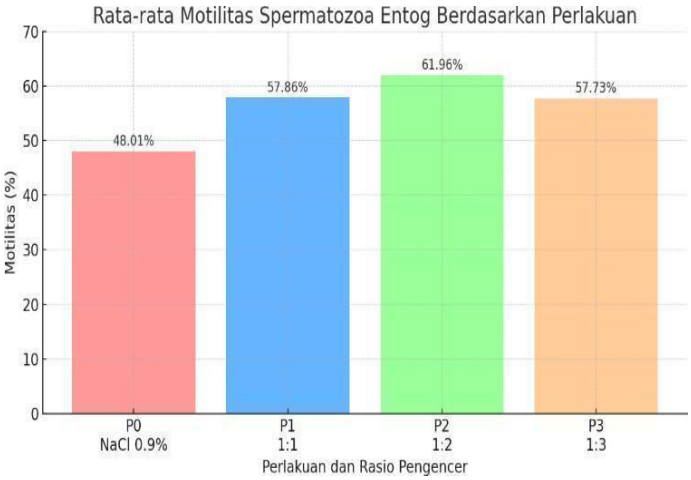

Figure 1: Average motility of duck sperm based on treatment.

The graph shows that treatment T2 (1:2) produced the highest motility of duck spermatozoa at 61.96%, which means that the combination of sugarcane juice and citrate-egg yolk at this ratio is most effective in maintaining active sperm movement. Treatments T1 (1:1) and T3 (1:3) showed moderate motility and were not significantly different from each other. While T0 (NaCl 0.9%) as the control produced the lowest motility (48.01%), indicating that physiological solution without additional nutrients is less able to maintain sperm quality. Thus, T2 is the best treatment to improve the motility of duck spermatozoa.

Table 1: Average motility and viability of duck spermatozoa per treatment.

Treatment	Motility (%)	Viability (%)
P0	48.01	66.89
P1	57.86	76.66
P2	61.96	80.01
P3	57.73	75.86

The addition of sugarcane juice extract and citrate-egg yolk as a natural diluent was shown to increase the motility and viability of duck spermatozoa. The best treatment was obtained in the combination of 1:2 (P2), because it provides an optimal balance between energy sources (glucose and fructose from sugar cane) and membrane protectors (lecithin from egg yolk).

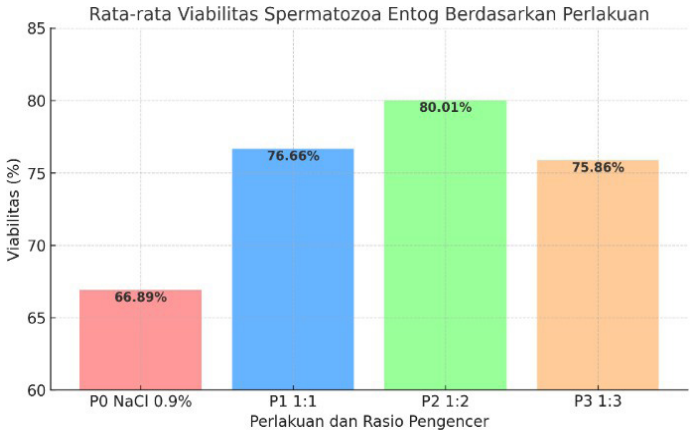

Figure 2: Average viability of duck sperm based on treatment.

Average viability of duck spermatozoa by treatment. This graph shows that treatment T2 (1:2) gave the highest viability (80.01%), followed by T1 and T3, while T0 (control) produced the lowest viability (66.89%). This proves that the combination of sugarcane juice and egg yolk-citrate can significantly increase sperm viability.

Table 2: DMRT test results motility.

Treatment	Average	Notation
T2	61.96	a
T1	57.86	b
T3	57.73	b
T0	48.01	c

Table 3: DMRT test results viability.

Treatment	Average	Notation
T2	80.01	a
T1	76.67	ab
T3	75.86	ab
T0	66.89	b

As reinforcing evidence in this study, there is a statistical analysis test attached to the following table presenting the results of the DMRT test for motility and viability that shows the important role of natural diluents in maintaining sperm quality. This combination effectively maintains sperm motility and viability, making it a cheap, readily available, and efficient alternative diluent for local poultry artificial insemination programs. Increased motility indicates the right combination to provide a protective effect on sperm movement during *in vitro* storage, the content of sugar water given contains glucose, fructose and sucrose compounds so that it can be utilized as an energy source for spermatozoa. While the content of egg yolks has lipoprotein and phospholipite compounds that can protect sperm membranes from damage, besides that the citrate compounds contained in egg yolks function as anticogulants and metal ion binders when sperm are damaged.

The addition of sugarcane juice extract and citrate-egg yolk as a natural diluent was shown to increase the motility and viability of duck spermatozoa. The best treatment was obtained in the combination of 1:2 (P2), because it provides an optimal balance between energy sources (glucose and fructose from sugar cane) and membrane protectors (lecithin from egg yolk) to determine the performance of diluents in affecting motility and viability of various treatments is attached to the bar chart as follows.

The graph shows that treatment T2 (1:2) produced the highest motility of duck spermatozoa at 61.96%, which means that the combination of sugarcane juice and citrate-egg yolk at this ratio is most effective in maintaining active sperm movement. Treatments T1 (1:1) and T3 (1:3) showed moderate motility and were not significantly different from each other. While T0 (NaCl 0.9%) as the control produced the lowest motility (48.01%), indicating that physiological solution without additional nutrients is less able to maintain sperm quality. Thus, T2 is the best treatment to improve the motility of duck spermatozoa. In addition, the performance of the viability diluent was as follows.

Average viability of duck spermatozoa by treatment. This graph shows that treatment T2 (1:2) gave the highest viability (80.01%), followed by T1 and T3, while T0 (control) produced the lowest viability (66.89%). This proves that the combination of sugarcane juice and egg yolk-citrate can significantly increase sperm viability. As reinforcing evidence in this study, there is a statistical analysis test attached to the following table presenting the results of the DMRT test for motility an, these results are in accordance with theory and previous research (Tanii *et al*., 2022) that shows the important role of natural diluents in maintaining sperm quality. This combination effectively maintains sperm motility and viability, making it a cheap, readily available, and efficient alternative diluent for local poultry artificial insemination programs. Increased motility indicates the right combination to provide a protective effect on sperm movement during *in vitro* storage, the content of sugar water given contains glucose, fructose and sucrose compounds so that it can be utilized as an energy source for spermatozoa. While the content of egg yolks has lipoprotein and phospholipite compounds that can protect sperm membranes from damage, besides that the citrate compounds contained in egg yolks function as anticogulants and metal ion binders when sperm are damaged. This research is the latest innovation to develop a sperm diluent formulation with a local-based, environmentally friendly system.

CONCLUSIONS

This study showed that the addition of sugarcane juice extract combined with citrate-egg yellow as a natural diluent was able to improve the quality of duck spermatozoa. The use

of the combination of diluents is proven to have an effect on increasing the motility and viability of spermatozoa significantly compared to the control treatment. The best treatment of motility and viability of duck spermatozoa was obtained in the combination of sugarcane juice diluent and egg yolk-citrate in a ratio of 1:2 (P2). This treatment gave a motility of 61.96% and viability of 80.01%, which was significantly different from the other treatments ($p<0.05$). This combination was able to provide energy and protect the integrity of the spermatozoa membrane, thus effectively improving the quality of the sperm. Thus, P2 natural diluent can be recommended as a significant alternative in supporting the success of artificial insemination programs.

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NOVELTY STATEMENT

This study was conducted to evaluate the effectiveness of a combination of sugarcane juice and egg yolk citrate as a natural diluent on the quality of entog spermatozoa, particularly motility and viability parameters. This study is expected to provide a more applicable diluent alternative for farmers in supporting the success of artificial insemination in local poultry.

AUTHOR'S CONTRIBUTION

SA: Conceptualized and designed the experiments. AT and PEP: Carried out the experimental work. YP and ASH: Analysed the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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