

THESIS

**SUPPLEMENTATION OF GREEN TEA EXTRACT IN
AN EGG-YOLK CITRATE SKIMMED MILK
EXTENDER ON *SAPUDI* RAM'S SPERMATOZOA
QUALITY AT A COLD TEMPERATURE (5°C)**



By

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UNIVERSITAS AIRLANGGA
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CITRATE SKIMMED MILK EXTENDER ON *SAPUDI* RAM'S
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Thesis

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by

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DECLARATION

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Supplementation of Green Tea Extract in an Egg-Yolk Citrate Skimmed Milk Extender on *Sapudi* Ram's Spermatozoa Quality at a Cold Temperature (5 °C)

has never been submitted to obtain a bachelorate degree in another higher education and to my knowledge, there is not a piece of writing or opinion that has been written or published by other people except for what has been clearly stated and cited in reference.

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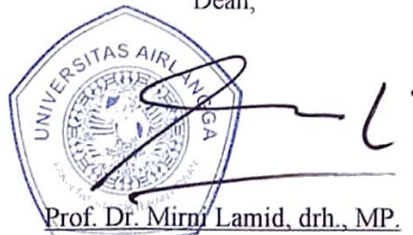
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SUMMARY

Although egg-yolk skimmed milk extender has been able to maintain fertile spermatozoa effectively upto day 4, there is a study by Bustamante-Filho et al. (2009) who found that antioxidant enzymes of the extended semen decreases drastically lower than fresh/raw semen. There was a decrease of 64% for catalase, 43% for glutathione peroxidase and 78% for superoxide dismutase. This means an addition of exogenous antioxidant into the extender can be observed to compensate for the high loss of it to enhance the efficacy of semen extender.

The addition of green tea extract can compensate the loss of antioxidant properties. The higher the dose (to a certain degree), the better the ability and this is due to its green tea extract's antioxidant properties such as polyphenol and flavonoid antioxidative properties, buffer from citrate and skimmed milk, and a cold-shock bumper from egg yolk towards spermatozoa's quality before storing for artificial insemination. It is vital to determine if the spermatozoa are categorized as good quality spermatozoa.

Semen samples were pooled of a healthy, 30 kg *Sapudi* ram. The sample collection is done 6 times using 4 treatments (Gill *et al.*, 2011). The type of semen that qualifies motility of $\geq 70\%$, viability of $\geq 70\%$, and mass motility ($\geq 2+$) are diluted in treatment groups and evaluated of their motility, viability, plasma membrane integrity every 24 hours until motility reaches 40%. After data has been collected, it is processed using

SPSS One-Way Analysis of Variant (ANOVA) which then can be further processed with Tukey test if the difference of data is significant (5%).

The results of the present study demonstrated a significant effect of added green tea extract on spermatozoa motility, viability, and plasma membrane integrity ($p < 0.05$) compared to non GTE-treated group. Further processing using Tukey HSD (5%) Post Hoc test shows the supplementation of 0.20% green tea extract in EYCSME (T3) resulted in a low maintenance of spermatozoa quality compared to EYCSME with no green tea extract supplementation, hence it is the best group.

The explanation behind it is due to green tea extract which acts as a protection against lipid peroxidation through its polyphenol which chelates oxidative agent by increasing antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. These lipids susceptibility to ROS is suppressed as the antioxidant is settled with lipids, resulting in a still high percentage of viability and maintenance of motility and plasma membrane integrity.

It can be concluded that supplementation of green tea extract in an egg-yolk citrate skimmed milk can maintain motility, viability, and plasma membrane integrity of *Sapudi* ram spermatozoa upto a 5-day storage. In terms of *Sapudi* semen preservation and the quality of refrigerator-preserved *Sapudi* ram semen, it is still eligible for artificial insemination (AI) up to 5 days (96 hours). However, further study should be directed to study the efficacy of these semen extenders processed as a frozen semen extender.

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Tanti Amarta Putri

ABSTRACT

The research purpose is to find out how supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affects motility, viability, and plasma membrane integrity of *Sapudi* ram's spermatozoa at a cold temperature (5°C). This type of research is an experimental research with diluted *Sapudi* ram semen sample being observed every 24 hours until motility reaches 30%; repeated 6 times. Smear preparations followed by calculating each parameter using formula were finished, analysed using One-way ANOVA, and further tested using Tukey post-hoc test if $p < 0.05$ (Mustofa *et al.*, 2021). Green tea extract-treated groups had a real significant on spermatozoa motility, viability, and plasma membrane integrity ($p < 0.05$). The results showed that the T3 had the highest means hence the best group (0.20% green tea extract in egg-yolk citrate skim milk extender (EYCSME)). Effect of T3 on day 1 storage motility, viability, and plasma membrane integrity is $84.17\% \pm 0.91$, $86.11\% \pm 2.33$, and $65.39\% \pm 2.71$. The lowest percentage of motility, viability, and PMI was obtained from the no added green tea extract in EYCSME respectively being $37.22\% \pm 8.61$, $62.94\% \pm 3.11$, and $29.77\% \pm 4.01$. This study concluded the supplementation of green tea extract in an EYCSME can maintain *Sapudi* ram's spermatozoa quality at a cold temperature (5°C). The dose of 0.20% gives a better effect in maintaining motility, viability, and plasma membrane integrity than lower concentrations in the long span of 5 days at 5°C.

Keywords: *Sapudi* ram; spermatozoa quality; egg-yolk citrate skimmed milk; green tea extract; EGCG

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ABBREVIATIONS AND SYMBOLS

g	: Gram
AI	: Artificial Insemination
ATP	: Adeno-Triphosphate
Ca ²⁺	: Cytosolic free calcium
c-AMP	: Cyclic Adenosin Monophosphate
Cu	: Cuprum
DNA	: Deoxyribonucleic Acid
EGCG	: Epigallocatechin gallate
EYCSM	: Egg-Yolk Citrate Skimmed Milk
Fe	: Ferrum (Iron)
GSH	: Glutathione peroxidase
GTE	: Green Tea Extract
H ₂ O ₂	: Hydrogen peroxide
HO	: Hydroxyl radical
HOST	: Hypo-Osmotic Swelling Test
IU	: International Units
Lab	: Laboratory
LDL	: Low Density Lipoprotein

LPO	: lipid peroxidation
mg	: Miligram
mL	: Mililiter
mm ³	: cubed millimeter
NADH	: Nicotinamide Adenine Dinucleotide
NP	: Nonprogressive
O ²⁻	: Superoxide anion
pH	: Potential Hydrogen
PMI	: Plasma membrane integrity
PR	: Progressive Motility
PUFA	: Polyunsaturated Fatty Acid
ROS	: Reactive Oxygen Species
SOD	: Superoxide dismutase
Zn	: Zinc
°C	: Degree Celcius
≥	: More than/equal to
≤	: Less than/equal to
%	: Percent