



Total Bacteria of Post-Thawing Boer Buck Semen with Addition of Sweet Orange Essential Oil to Tris Yolk Extender

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Authors' contributions

This work was carried out in collaboration among all authors. Author SAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZU, Jaswandi and Hendri managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the effect of the addition of sweet orange essential oil to the extender (tris yolk extender without the addition of antibiotics/antibacterial) to total bacteria Boer Buck frozen semen.

Study Design: Randomized Block Design.

Place and Duration of Study: Sample: Laboratory Reproduction of Loka Penelitian Kambing Potong Sei Putih Indonesia, between January and March 2019.

Methodology: The research procedure starts with the preparation of semen extender, collection of fresh semen, dilution of semen, equilibration, freezing of semen, and thawing. This research obtained conducted using a Randomized Block Design consisting of 5 treatment levels and five replications. Semen storage using 3 Boer buck, which done for three days. As a treatment is the addition of sweet orange essential oil as much as (P0) 0%, (P1) 0,25%, (P2) 0,5%, (P3) 0,75% and (P4) 1% on the tris yolk extender. The observed variables was total bacteria evaluated before freezing and after freezing (Post-Thawing).

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Results: The results showed that the addition of sweet orange essential oil had a very significant effect ($P < 0.01$). The results of adding sweet orange essential oil to the extender (Post-Thawing) were 78.6×10^2 CFU (P0), 76×10^2 CFU (P1), $73,2 \times 10^2$ CFU (P2), 71×10^2 CFU (P3) and 68.6×10^2 CFU (P4).

Conclusion: The best values the total bacteria 68.6×10^2 CFU (P4). It can conclude that the best result of adding sweet orange essential oil to tris yolk extender is (P4) 1%.

Keywords: Boer Buck; post-thawing; sweet orange essential oil; total bacteria.

1. INTRODUCTION

Bibit ternak berkualitas baik sangat mempengaruhi keuntungan peternak [1]. The quality of the frozen semen Boer buck is terrible because of high bacterial development. Bacterial contamination can occur, starting from the collection of fresh semen until the process of making frozen semen [2]. According to the International Committee for Animal Recording (ICAR), the maximum total bacterial requirement allowed is 5×10^3 CFU / ml [3]. The addition of antibiotics to the extender commonly done to inhibit and kill bacteria in frozen semen [4]. However, the current use of antibiotics has begun to abandoned and replaced with natural ingredients.

To reduce the total bacteria in the frozen semen Boer buck can be done by adding ingredients that contain antibacterial to the extender. Sweet orange essential oil is a natural ingredient that can be used as an antibacterial because it can inhibit and be active against bacteria [5]. The sweet orange essential oil contains the main components, including limonene, linalool, pinene, and oktanal [6]. Limonene and linalool are toxic to bacteria [5]. The sweet orange essential oil also contains antioxidants [7]. Antioxidants can reduce damage caused by cold shock in spermatozoa [8]. The addition of sweet orange essential oil on the tris yolk extender expected to reduce total bacteria and improve the quality of frozen semen Boer Buck.

2. MATERIALS AND METHODS

The research carried out in the laboratory by adding sweet orange essential oil as an antibacterial source to the quality of the post-thawing Boer buck semen. Semen processing starts from a collection of fresh semen, dilution semen, equilibration, semen freezing, and thawing. Microbiological culture 22.5 g Plate Count Agar (Merck®) dissolved in 1000 ml distilled water until homogeneous. Heated using a hot plate, then sterilized using an autoclave at

121°C and 15 psi pressure for 15 minutes. 1 ml sample was put into a test tube containing 9 ml of 0.1% peptone solution, then homogenized with vortex until the fourth dilution. Samples were grown on Petri dishes containing frozen PCA media using the spread method. Samples were incubated in an incubator at 37°C for 24 hours, and the growing bacterial colonies counted using a Quebec Colony Counter.

Materials used were fresh semen Boer buck, sweet orange essential oil (using sweet orange peel and steam distilled), nutrient agar and nutrient broth (to breed the bacteria in Boer goat frozen semen), Tris (hydroxymethyl) aminomethane (3.32 g), Citric Acid (1.86 g), Fructose (1.37 g), Glycerol (6ml), egg yolk (20 ml), aqua dest (100 ml), eosin 2% (to color the dead spermatozoa), liquid nitrogen, incubator, autoclave, oven, petri dish, beaker glass, cover glass, and denominator. All ingredients use the brand merck.

Statistical analysis using a Randomized Block Design consisting of 5 treatment levels and five replications. Experimental group is a holding of fresh semen using 3 Boer buck once every three days, those aged 2-4 years with body weights ranging from 45-50 kg. As treatment is the addition of sweet orange essential oil to the tris yolk extender. Duncan's test tested differences between treatments. The treatments observed:

- P_0 = Tris Yolk Extender + Sweet Orange Essential Oil 0%
- P_1 = Tris Yolk Extender + Sweet Orange Essential Oil 0.25%
- P_2 = Tris Yolk Extender + Sweet Orange Essential Oil 0.5%
- P_3 = Tris Yolk Extender + Sweet Orange Essential Oil 0.75%
- P_4 = Tris Yolk Extender + Sweet Orange Essential Oil 1%

The parameters observed were the evaluation of semen before freezing and after freezing, namely:

2.1 Total Bacteria

The procedure for calculating total bacterial colonies according to the standard plate count method, using Plate Count Agar media, which diluted four times [9]. The formula calculates bacteria:

$$\text{CFU/ml} = \text{TKB} \times \frac{1}{fp} \times \frac{1}{s}$$

Description:

TKB = Total Bacterial Colonies
fd = Factor Diluent (10^{-2})
S = Sample (0,25)

3. RESULTS AND DISCUSSION

3.1 Total Bacteria

The results of the study of the total bacteria of Boer Buck frozen semen before freezing and after freezing can see in Table 1. The best research results obtained in the treatment of 1% (P_4) with a total bacteria of $68,6 \times 10^2$ CFU/ml and worst in the treatment of 0% (P_0) with a total

bacteria of $78,6 \times 10^2$ CFU/ml. All treatments in this study did not meet the standard as an extender because the minimum total bacterial requirements in frozen semen, according to the International Committee for Animal Recording (ICAR) was 5×10^3 CFU/ml [3]. It can conclude that the use of sweet orange essential oil as an extender is not enough to reduce the total bacteria in frozen semen Boer buck. Variance analysis results showed that the effect of adding sweet orange essential oil was significantly different ($P < 0.01$) on total bacteria in frozen semen Boer buck.

The reproductive tract is not a sterile environment. The frozen semen trade worldwide requires bacterial control. Antibiotics have used to control the bacteria contained in semen, and research is needed to find out whether the material is toxic to spermatozoa or not [10]. Microorganisms, especially bacteria, can affect fertility [11]. Fresh semen ejaculated from commercial rams often contains bacterial flora with concentrations up to 10^8 CFU/ml [12]. Bacterial contamination occurs due to infection of the reproductive tract and the entry of microorganisms during collection, processing, and storage [2].

Table 1. The effect of supplementation of sweet orange essential oil on tris yolk extender on the total bacteria of boer buck semen before freezing and after freezing

Parameter	Treatment	Observation	
		Before Freezing	After Freezing
Total Bacteria (x 10^2 CFU/ml)	P_0	(84.6±1,52) ^a	(78.6±1,14) ^a
	P_1	(82.8±2,59) ^b	(76±1,58) ^b
	P_2	(81.2±1,92) ^c	(73.2±1,92) ^c
	P_3	(79±1,58) ^d	(71±1,58) ^d
	P_4	(77±1,58) ^e	(68.6±2,70) ^e

Note: Different superscripts in each variable column shows very significant differences ($P < 0.01$)

Table 2. Total value of bacteria in boer goat semen after freezing with the addition of sweet orange essential oil to on tris yolk extender

Group	Treatment				
	P_0	P_1	P_2	P_3	P_4
1	77	75	72	70	65
2	78	76	71	69	67
3	79	74	74	71	69
4	79	77	73	73	70
5	80	78	76	72	72
Total	393	380	366	355	343
Average	(78.6±1,14) ^a	(76±1,58) ^b	(73.2±1,92) ^c	(71±1,58) ^d	(68.6±2,70) ^e

Note: Different superscripts in each variable column shows very significant differences ($P < 0.01$)

The highest total bacteria show a population of microorganisms that grow very fast. The effect of bacterial contamination on reproductive organs is huge [13]. The mechanism of bacterial pathogenicity mechanically based on the ability of bacteria to multiply in large numbers. Bacteria can form colonies and attach themselves to the mucosa. It can damage the tissue and structure of the reproductive organs. It also can kill spermatozoa, which will fertilize the ovum so that fertilization fails. Apabila hal ini terjadi, tidak akan terjadi fertilisasi sehingga akan merugikan peternak [14].

During semen collection, it is difficult to avoid contamination with bacteria from the environment [2]. To minimize bacterial contamination is to increase hygiene measures during semen collection and processing. Dilution of fresh semen with a sterile extender will further reduce the concentration of contaminants [15]. Bacterial contamination of semen can occur at any time during the insemination process, especially during semen storage. Several types of bacteria have found so that fresh semen is often contaminated by bacteria that can survive temperatures up to -196°C in liquid nitrogen [16].

Semen plasma contains antimicrobial properties that can reduce bacterial growth. Proteins and peptides contained in semen plasma are active against antimicrobials, including *E. coli* and *Pseudomonas* spp [17]. However, it still needs to add antibacterial ingredients to minimize bacterial growth further. The flavonoid content in sweet orange essential oil inhibits gram-positive and gram-negative bacteria, which has been tested on *Escherichia coli* and *Staphylococcus aureus* [18]. Meanwhile, sweet orange essential oil contains significant components such as limonene and linalool, which are toxic to bacteria [5]. The content of limonene in the sweet orange essential oil is very high, at 82.02% [6].

4. CONCLUSION

The addition of 1% sweet orange essential oil to Tris Yolk extender can lowered the total bacteria on Boer buck frozen semen. However, it still needs to be done a combination of sweet orange essential oil with other ingredients that contain antibacterial, because the total bacteria present in Boer buck frozen semen is above the minimum standard of the International Committee for Animal Recording (ICAR).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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