**Original Research Article**

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

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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).

**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 x 10² CFU (P0), 76 x 10² CFU (P1), 73.2 x 10² CFU (P2), 71 x 10² CFU (P3) and 68.6 x 10² CFU (P4).

**Conclusion:** The best values the total bacteria 68.6 x 10² 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**

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 [1]. According to the International Committee for Animal Recording (ICAR), the maximum total bacterial requirement allowed is 5 x 10³ CFU / ml [2]. The addition of antibiotics to the extender commonly done to inhibit and kill bacteria in frozen semen [3]. 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 [4]. The sweet orange essential oil contains the main components, including limonene, linalool, pinene, and oktanal [5]. Limonene and linalool are toxic to bacteria [4]. The sweet orange essential oil also contains antioxidants [6]. Antioxidants can reduce damage caused by cold shock in spermatozoa [7]. 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. MATERIAL 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. The research procedure starts from a collection of fresh semen, dilution semen, equilibration, semen freezing, and thawing.

Materials used were fresh semen Boer buck, sweet orange essential oil, nutrient agar, nutrient broth, Tris (hydroxymethyl) aminomethane (3.32g), Citric Acid (1.86g), Fructose (1.37g), Glycerol (6ml), egg yolk (20ml), aqua dest (100ml), eosin 2%, liquid nitrogen, incubator, autoclave, oven, petri dish, beaker glass, cover glass, and denominator.

This research conducted using a Randomized Block Design consisting of 5 treatment levels and five replications. The group is a holding of fresh semen using 3 Boer buck once every three days. 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:

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

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

**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 [8]. The formula calculates bacteria:

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

Description:

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

3. RESULTS AND DISCUSSION

**Total Bacteria**
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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Before Freezing</th>
<th>After Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>(84.6±1.52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(78.6±1.14)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>(82.8±2.59)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(76±1.58)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Bacteria</td>
<td>P2</td>
<td>(81.2±1.92)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(73.2±1.92)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(x 10&lt;sup&gt;2&lt;/sup&gt;CFU/ml)</td>
<td>P3</td>
<td>(79±1.58)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>(71±1.58)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>(77±1.58)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(68.6±2.70)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

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<sub>4</sub>) with a total bacteria of $68.6 \times 10^2$CFU/ml and worst in the treatment of 0% (P<sub>0</sub>) 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 $5 \times 10^3$CFU/ml [2]. 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 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 [9]. Microorganisms, especially bacteria, can affect fertility [10]. Fresh semen ejaculated from commercial rams often contains bacterial flora with concentrations up to $10^8$ CFU/ml [11]. Bacterial contamination occurs due to infection of the reproductive tract and the entry of microorganisms during collection, processing, and storage [1].

During semen collection, it is difficult to avoid contamination with bacteria from the environment [1]. 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 [12]. Bacterial contamination of semen can occur at any time during the insemination process, especially during semen storage. The reproductive tract is not a sterile environment. Several types of bacteria have found so that fresh semen is often contaminated by bacteria that can survive temperatures up to $-196^\circ$C in liquid nitrogen [13].

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 [14]. However, it still needs to add antibacterial ingredients to minimize bacterial growth further.
The highest total bacteria show a population of microorganisms that grow very fast. The effect of bacterial contamination on reproductive organs is huge [15]. 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.

The flavonoid content in sweet orange essential oil inhibits gram-positive and gram-negative bacteria [16]. Meanwhile, sweet orange essential oil contains significant components such as limonene and linalool, which are toxic to bacteria [4]. The content of limonene in the sweet orange essential oil is very high, at 82.02% [5].

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).

REFERENCES